UNIVERSITY OF ALBERTA

THE EFFECT OF AGGREGATION ON UV INACTIVATION OF MICROORGANISMS IN FILTERED WATER

by

HEEJIN KANG ©

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ABSTRACT

UV is rapidly becoming the technology of choice for providing additional protection against microorganisms in drinking water treatment plants. However, microorganisms may be attached to or enmeshed within particles present in filtered drinking water and this may impact the efficiency of UV disinfection. In this study, samples of untreated water from three rivers in Alberta were spiked with surrogate microorganisms (*Bacillus subtilis* spores and MS2 coliphage) and were subjected to simulated drinking water treatment conditions. The water was then exposed to controlled doses of UV light in the laboratory. The results of this study suggest that a fraction of *Bacillus subtilis* and MS2 coliphage that broke through the filter media were aggregated. The effect of aggregation phenomenon on UV disinfection was independent of the water source and the season during which the water collected, and was mainly determined by the coagulation conditions and the type of microorganisms.

DEDICATION

To my wife, Hyechung Cho, for her love and her support and to my parents, Bongho Kang and Younghee Kim, for the sacrifices they made for me to complete this degree.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
ATCC	American Type Culture Collection
AWWA	American Water Works Association
BR	Bow River
B+UV	Blended before UV exposure
CFU	Colony Forming Units
DI water	De-ionized water
DNA	Deoxyribonucleic Acid
df	Degrees of Freedom
H+UV	Homogenized before UV exposure
ID	Internal Diameter
IS	Intergrating Sphere
JCR	Jointed Confidence Region
Log	Logarithm
LT2ESWTR	Long Term 2 Enhanced Surface Water Treatment Rule
MS	Mean Square
NS	Not Significant
NSR	North Saskatchewan River
NTU	Nephelometric Turbidity Unit
PBS	Phosphate Buffered Saline
RDR	Red Deer River
PFU	Plaque Forming unit
PVC	Polyvinyl Chloride
RNA	Ribonucleic Acid
RPM	Revolutions Per Minute
S	Significant
SRT	Solids retention time
SS	Sum of squares
T4	Bacteriophage Type T4

t-test	Statistical 'student t' test
UV	Ultraviolet
USEPA	United States Environmental Protection Agency
UV+H	Homogenized after UV exposure
UV+B	Blended after UV exposure

LIST OF SYMBOLS

a	Absorption coefficient
b	The estimate of the model parameter using least square method
c	Speed of light
d	Sample depth
E	Radiometer reading at the center of the dish
E_{avg}	Average germicidal fluence rate
h	Planck's constant
H_0	UV fluence
I	Irradiance
k	Rate constant, linear
k_m	Rate constant, multi-target
k_s	Rate constant, series event
L	Path length
n	Number of sample points
n_c	Number of targets in the multi-target model
N	Concentration of infectious microorganisms after exposure to UV light
N_0	Concentration of infectious microorganisms before exposure to UV light
p-value	Probability of a Type I error
Q	Energy of radiation
t	Time
X	The derivative matrix from Taylor expansion of the model
X'	The rotated matrix of X

LIST OF GREEK SYMBOLS

λ	Wavelength
σ	The standard deviation of the measurement.

1. INTRODUCTION

1.1 BACKGROUND

One of the objectives of the Alberta provincial "Water for Life Strategy" is to ensure provision of safe and secure drinking water for all Albertans. One way to achieve this is through selection of appropriate water treatment technology and rational design criteria. Ultraviolet light has been shown to be effective for inactivating waterborne protozoan pathogens such as *Cryptosporidium parvum*, and *Giardia spp.* although viruses are somewhat more resistant. Due to low cost and ease of retrofit, UV is rapidly becoming the technology of choice for providing additional protection against microorganisms in drinking water treatment plants in Alberta. New regulations in the US EPA Long term 2 Surface Water Treatment Rule state that a minimum UV dose of 12 mJ/cm^2 is required for 3 log reduction of *C. parvum*. New Alberta water treatment standards are expected to adopt similar criteria.

Microorganisms associated with particulate matter may be protected to some extent from exposure to UV radiation and may thus exhibit an apparent increase in resistance to UV even thought the UV dose applied to the water is appropriate. For example, it is has been shown that a fraction of the coliform bacteria population in secondary municipal wastewater is harboured within bacterial floc particles and is thus shielded from UV exposure. This shielding results in tailing in the UV-dose inactivation curve and some degree of shielding are believed to be governed by floc particle size. It is unclear if microorganisms present in filtered drinking water would be shielded in a similar way since the nature of the particulate matter and the interaction with microorganisms is likely to be entirely different than in secondary

wastewater. The effect of particulate matter on UV inactivation of microorganisms in drinking water has been studied to a limited extent mainly by seeding water samples with purified preparations of the microorganisms and then exposing the suspension to controlled doses of UV radiation using a collimated beam apparatus and standard procedures. Using this approach, *Crytosporidium* oocysts seeded into filter backwash water samples at high concentration were found to be readily inactivated by UV. Similarly, UV inactivation of MS2 bacteriophage seeded into unfiltered and filtered drinking water was unaffected by turbidity as long as water absorbance was accounted for in the determination of UV dose. An increase in turbidity from 0.25 to 20 NTU was associated with a statistically significant but modest reduction in UV inactivation of *Crytosporidium* oocysts and *Giardia cysts* seeded into unfiltered water. On the other hand, the degree of tailing in the UV inactivation curves of *Bacillus subtilis* spores was found to be related to microorganism aggregation. Ultraviolet inactivation of *Giardia* cysts increased when bacterial and colloidal debris were better removed from the cyst preparations prior to UV exposure, and colloidal humic acid particles were found to have a statistically significant impact on UV inactivation of MS2 coliphage seeded into synthetic water while Kaolin clay particles had little effect. Under the correct conditions colloidal organic matter may, therefore, absorb onto microorganism surfaces, thereby preventing penetration of UV (Templeton et al., 2005).

One of the limitations of UV disinfection criteria is that they are based largely on the outcomes of laboratory studies in which samples of de-ionized water or filtered

drinking water were seeded with purified parasite preparations and exposed to well-defined UV doses in collimated beam experiments.

1.2 HYPOTHESIS

The hypothesis of this research is that microorganisms present in drinking water that has been pre-treated by coagulation, flocculation, sedimentation and granular-media (anthracite-sand) filtration are likely to be aggregated with other microorganisms or inert particulate matter due to the nature of the upstream processes. The UV inactivation characteristics of these aggregated microorganisms are, therefore, likely to be different than that indicated by laboratory studies with mono-dispersed microorganisms. As a result, the level of public health protection provided by UV reactors located downstream of filtration in the treatment train could be overestimated. This is particularly relevant during periods of treatment plant upset, suboptimal coagulation and poor performance of the filtration process when coagulated microorganisms and particles may breakthrough the filter.

1.3 OBJECTIVES

The objective of this research was to further explore and better understand the phenomenon of microbial aggregation in filtered drinking water with the eventual goal of developing recommendations to the water treatment industry. The specific goals were to:

- Determine if the aggregation phenomenon is repeatable with other types of microorganisms and with different raw water sources. Since research using ***C.***

parvum is time-consuming and expensive, the range of variables that can be explored practically is very limited. In this research, greater use will be made of surrogate microorganisms as they are relatively easy and inexpensive to produce and to enumerate. Two microbial indicators representing a fairly broad spectrum of microorganism size and morphology were used: ***B. subtilis*** **spores and MS2 coliphage.** Three raw river waters were used: **North Saskatchewan River, Bow River and Red Deer River**.

- Determine if the extent of aggregation and the effect on UV inactivation can be predicted based on readily measurable filtration performance variables such as turbidity and particle concentration and size distribution. To achieve this goal, different coagulant doses were used to simulate different levels of turbidity and particle concentration in the filtered water (optimal, sub-optimal and poor). The level of aggregation and UV resistance may be correlated with measured turbidity or particle concentration.

1.4 OUTLINE OF THESIS

This thesis is organized into five chapters. Chapter 1 provides a background and describes study objectives and hypothesis. A review of relevant literature is presented in Chapter 2. Chapter 3 provides detailed information about the materials, experimental apparatus, procedures, and techniques used to accomplish the objectives of this thesis. Experimental results are presented in Chapter 4 and discussed in Chapter 5. Chapter 6 contains the conclusion and recommendations. The appendixes contain raw data, sample calculations and results of supporting experiments.

2. LITERATURE REVIEW

This literature review provides background information on the topics discussed in the subsequent chapters. The chapter begins with a review of the definition of ultraviolet (UV) light, mechanisms of UV inactivations, UV dose and UV dose-response effects or characteristics. Next is a description of the biodosimetry indicator organisms used in this study (*B. subtilis* spores and MS2 coliphage).

The remainder of the review is a discussion of how particle-associated microorganisms impact UV disinfection in water and wastewater treatment processes. This includes a description of ultraviolet disinfection processes and previous studies of the effect of particle-associated microorganisms. At the end of this chapter, the need for this research is discussed.

2.1 UV RADIATION

2.1.1 Definition of Ultraviolet Light

Ultraviolet (UV) is part of the electromagnetic spectrum. Only a small portion of electromagnetic radiation is visible to the human eye. Photochemical reactions can be driven by light wavelengths ranging from 100 to 1000 nm. Within this range lies UV (100 to 400 nm) and the visible light spectrum (400 to 700 nm). One of the most recognizable examples of a photochemical reaction is photosynthesis. In 1900, Max Planck quantified the energy of radiation by assuming that energy would be proportional to the frequency if the radiation were in discrete "chunks" or "packets". This relationship can be expressed as

$$Q = \frac{hc}{\lambda} \qquad \text{Equation 2.1}$$

where Q is the energy of one pho

ton (J), h is Planck's constant (6.6261×10^{-34} J•s), c is the speed of light (2.9979×10^{8} m/s) and λ is wavelength (m). The energy of a photon increases as its wavelength decreases. For this reason, UV light is the most studied spectrum for the investigation of photochemical reactions.

UV light can be classified into 4 ranges based on its effect on human skin as described in Table 2-1.

Table 2-1. The electromagnetic spectrum of UV light

TYPE	RANGE	COMMENT
UV-A	400 to 315 nm	Sometimes called near UV
UV-B	315 to 280 nm	Sometimes called medium UV
UV-C	280 to 200nm	Range to be considered in more detail in water disinfection
Vacuum UV	200 to 100nm	

UV-C is the most dangerous range because it is not completely absorbed by the atmosphere and is absorbed by RNA, DNA and proteins, which can result in cell mutations and death. UV-C has the smallest wavelength and the highest energy.

2.1.2 Mechanisms of UV Inactivation of Microorganisms

Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) are chain-like macromolecules that function in the storage and transfer of a cell's genetic information. These compounds generally comprise 5 to 15 percent of a cell's dry

weight, and effectively define the operations of a cell, particularly the type and quantity of enzyme production. The DNA molecule is considered to be the principal target of UV photons, and the primary component where significant biological effect, or damage, is incurred.

The monomeric units of the DNA and RNA are nucleotides. These all have three characteristics components: each has a nitrogenous heterocyclic base which can be either a purine or pyrimidine derivative; each contains a pentose sugar; and each has a molecule of phosphoric acid. There are four different deoxyribonucleotides which comprise the major components of DNA, differing only in their base components. Two are the purine derivatives adenine and guanine; the other two are pyrimidine derivatives cytosine and thymine. Similarly, four different ribonucleotides comprise the major components of RNA. As with the DNA, they contain the purine bases guanine and adenine; the pyrimidine bases are cytosine and uracil. Thus, thymine is characteristically present only in DNA, while uracil is normally present only in RNA.

Common disinfection chemicals like chlorine and ozone inactivate microorganisms by oxidation of cellular material. However, UV microbial reduction relies on an entirely different mechanism. The UV light is absorbed by cell nucleic acid, which is believed to cause dimerization of adjacent pyrimidine nucleobases (von Sonntag and Schuchmann, 1992). Both purine and pyrimidine bases undergo significant UV adsorption, but the rate of damage is much larger in pyrimidines. In DNA, thymine-thymine dimer formation dominates, because thymine has higher absorbance than cytosine in the germicidal UV range. The formation of both uracil-uracil and cytosine-cytosine dimers are common in RNA. Dimer formation prevents

DNA and RNA replication. Without replication, the microorganism cannot reproduce and therefore cannot infect.

DNA and RNA components of most living organisms absorb UV at wavelengths between 240 to 300 nm, with peak absorption at about 265nm (Harm, 1980). This UV absorbance peak coincides with the UV-C wavelengths which can be reliably produced with commercially available mercury vapor UV lamps. Because this range is more effective in inactivating living cells when compared to UV-A and UV-B, it is usually referred to as germicidal UV (Bolton, 1999; Harm 1980)

The germicidal efficiency curve closely matches the UV absorbance curve of major pyrimidine components of nucleic acids, as illustrated in Figure 2-1.

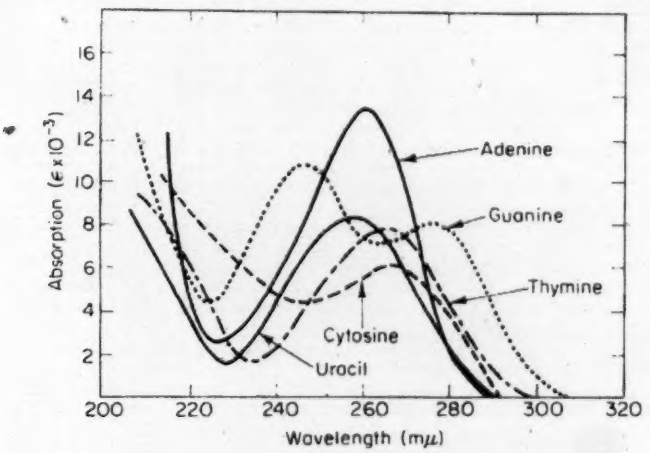

Figure 2-1. UV-C absorptivity of pyramidine bases.

(adapted from Jagger, 1967.)

Some organisms have natural defenses against mutations in their nucleic acid. Photo-reactivation is an enzyme based repair mechanism that allows microorganisms

to mend minor damage to their DNA. When the damaged organism is exposed to light between 310 to 490 nm, the enzyme DNA photolyase becomes activated and cleaves the dimers from the DNA. Light-independent repair mechanisms are also enzyme based. The presence of DNA repair mechanisms increase the minimum UV dose required to achieve a given level of microbial inactivation. The goal of microbial reduction using UV light is to damage the organism to a degree that DNA repair will not occur. High UV doses can directly cause cell death, but lower doses are adequate because replication is prevented.

2.1.3 UV Dose

UV dose is a measurement of the energy per unit area that is incident on a surface. UV dose is the product of the average fluence rate acting on a microorganism from all directions and the exposure time.

In a batch system such as a bench scale collimated beam test, the average intensity is determined mathematically. For collimated beam tests using a low-pressure lamp, the UV intensity measured by a radiometer, the UV absorbance of the water, the thickness of the water layer, the distribution of light across the water surface, and the reflection and refraction of light from the water surface all are considered in calculating the average intensity within the water sample.

The radiometer detector only provides a measure of the fluence rate incident on the water at the center of the beam. Several corrections are required to obtain the average irradiance in the water. These corrections are described in subsequent sections. The divergence factor value is most important, since this provides an

estimate of the average fluence rate to which each microorganism is exposed and is the basis on which the delivered fluence to a sample can be calculated (Bolton and Linden, 2003).

Suspended particles may influence the design and operation of UV treatment installations. The UV demand that water exerts is most often described by the absorption coefficient of the water (USEPA, 1996). By deflecting UV light away from the instrument's detector, the scattering properties of suspended particles can affect the validity of absorbance measurements made with a standard spectrophotometer. This then registers some of the transmitted light as attenuated light, which falsely increases the absorbance measurement (Linden and Darby, 1998). Because of scattering, reliance on standard absorbance measurements for UV reactor design may result in over-design or inefficient operation of UV treatment systems.

Fig. 2-2 illustrates the effect of UV absorbance and transmittance at 254 nm, 1 cm cuvette conducted by mounting an integrated spheres setup versus using a conventional spectrophotometer (Mamane-Gravetz and Linden, 2006).

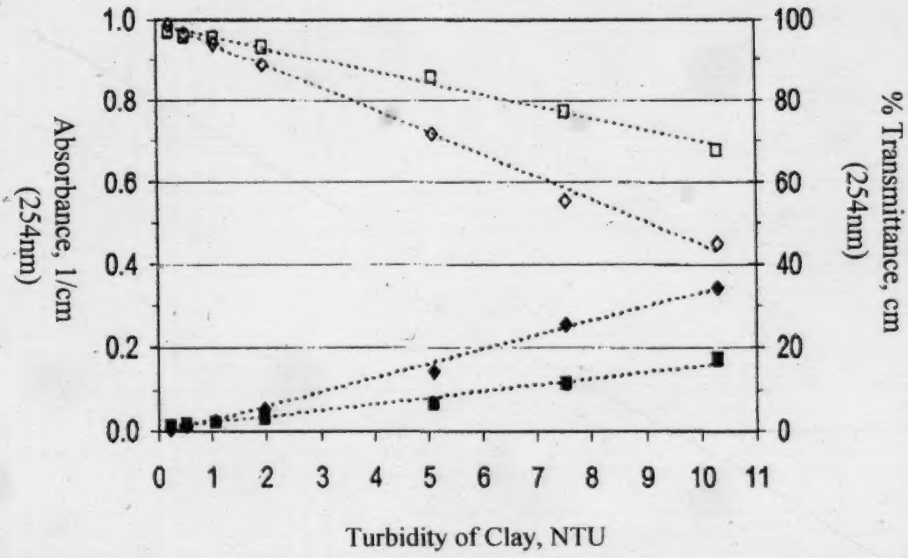

Figure 2-2. Comparison of direct and integrating sphere absorbance and transmittance measurements at 254nm of suspended clay particles as a function of turbidity. Samples are represented by a direct absorbance (◆), an IS absorbance (■), a direct UV transmittance (◇) and an IS UV transmittance (□). (adapted from Mamane-Gravetz and Linden, 2006)

Above values of 2 NTU clay particles, a difference between absorbance measurements is observable with falsely higher values of absorbance occurring with the use of the standard spectrophotometer. This difference in absorbance measurements increases with increasing turbidity of the water. Multiple scattering and interparticle interferences are of concern for highly concentrated particle samples and the interparticle interferences can become significant in the case of highly charged particles or particles with refractive indices close to 1. Therefore, for water

with turbidity > 3 NTU the use of direct conventional absorbance measurement in the calculations of fluence leads to overdosing of the UV system. Use of Integrating Sphere spectroscopy can overcome the problem with conventional spectroscopy in absorbance measurements of scattering suspensions (Mamane-Gravetz and Linden, 2006)

2.1.3.1 Reflection Factor

Whenever a beam of light passes from one medium to another, where the refractive index changes, a small fraction of the beam is reflected off the interface between the media. For a normally incident beam, the fraction reflected R is given by Frensel's Law (Meyer-Arendt, 1984). For air and water, the average refractive indices in the 200 to 300nm region are 1.000 and 1.372, respectively. Thus for these two media R=0.025, and the reflection Factor is (1-R) = 0.975, and represents the fraction of the incident beam that enters the water.

2.1.3.2 Petri Factor

Depending on the design of the bench scale apparatus, the irradiance will vary somewhat over the surface area of the liquid sample to be irradiated. The Petri Factor is defined as the ratio of the average of the incident irradiance over the area of the Petri dish to the irradiance at the center of the dish and is used to correct the irradiance reading at the center of the Petri dish to more accurately reflect the average incident fluence rate over the surface area. The Petri Factor may be determined by methodically scanning the radiometer detector (every 5mm) over the area of the Petri

dish, dividing the irradiance at each point by the center irradiance, and taking an average of these ratios. Because the detector sensor is wide, more accurate results are obtained with a partially blinded sensor, obtained by masking the sensor and thus reducing the area exposed to the light during the Petri Factor determination. Alternatively, for more accurate results a fiber optic probe can be used. A spreadsheet is used to allow these measurements to be made and to calculate the Petri Factor. In general, a well designed collimated beam apparatus should be able to deliver a Petri Factor of greater than 90% (Bolton and Linden, 2003).

2.1.3.3 Water Factor

If the water absorbs UV at the wavelengths of interest, then it is necessary to account for the decrease in irradiance arising from absorption as the beam passes through the water. The Water Factor is defined as:

$$WaterFactor = \frac{1-10^{-al}}{al\ \ln(10)} \qquad \text{Equation 2.2}$$

where a = decadic absorption (cm^{-1}) or absorption for a 1cm path length and l = vertical path length (cm) of the water in the Petri dish (Bolton and Linden, 2003).

2.1.3.4 Divergence Factor

The Divergence Factor is the average of this function over the path length L of the cell suspension.

$$Divergence\ Factor = \frac{L}{(L+x)} \qquad \text{Equation 2.3}$$

To be exact, the divergence and the water absorbance effects need to be considered together. However, for path lengths less than 5 cm, the errors involved in treating them separately are negligible.

2.1.3.5 Average Germicidal Fluence Rate

For a low pressure UV lamp for which the only significant emission is at 253.7nm, only the above four corrections are necessary to obtain the average germicidal fluence rate E_{avg} (W m^{-2}) in the water. Thus, for a low pressure UV lamp, E_{avg} is given by:

$$E_{avg} = E \times \text{Petri Factor} \times \text{Reflection Factor} \times \text{Water Factor} \times \text{Divergence Factor} \qquad \text{Equation 2.4}$$

where E = radiometer reading at the center of the dish and at a vertical position so that the calibration plane of the detector head is at the same level as the top of the solution. The UV dose can be determined in a batch system by multiplying the calculated average intensity by the specific exposure time.

2.1.4 UV Dose-Response

The response of microorganisms to UV light is calculated by determining the concentration of infectious microorganisms before and after exposure to a measured UV dose and applying Equation 2.5.

$$Log\ Inactivation = \log_{10} \frac{N_0}{N} \qquad \text{Equation 2.5}$$

where

N_0 = Concentration of infectious microorganisms before exposure to UV light

N = Concentration of infectious microorganisms after exposure to UV light

UV dose-response relationships can be expressed as either the proportion of microorganisms inactivated (log inactivation, results in a dose-response curve with a positive slope) or the proportion of microorganisms remaining (log survival, results in a dose-response curve with a negative slope) as a function of UV dose. The proportion of microorganisms remaining and the log inactivation are typically shown on a logarithmic (base 10) scale, while the UV dose is typically shown on a linear scale.

Although several approaches may be used to measure microbial dose-response, the bench-scale collimated beam test has evolved as the customary method because conditions can be carefully controlled, allowing for accurate and repeatable determination of UV dose. Accurate determination of UV dose is beneficial for developing meaningful relationships between UV dose and microbial response.

Figure 2-3 presents examples of UV dose-response curves. In general, the UV dose-response of disperse microorganisms follows first order inactivation. However, some microorganisms are slower to respond, producing a shoulder at low UV doses followed by near-linear inactivation. The UV dose-response is generally independent of how the germicidal UV light is produced, UV absorbance, temperature, and pH.

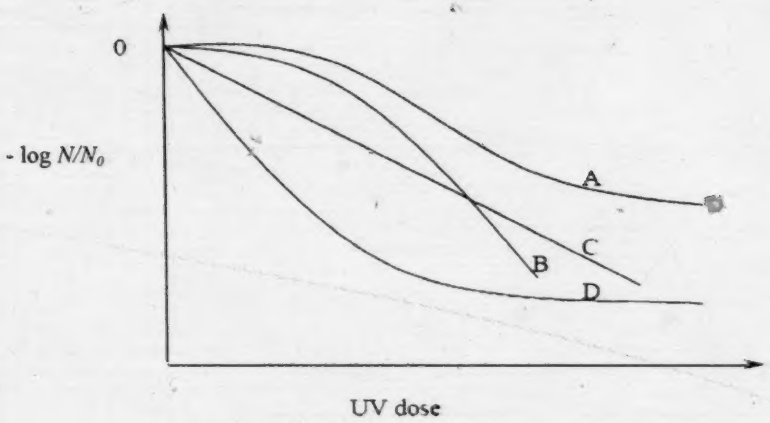

Figure 2-3. Types of microbial inactivation or survival curves exhibiting: shoulder and tailing (A), shoulder (B), linear rate (C) and tailing (D).

Functional representations of a linear and a shouldered survival curve are given in Equations 2.6 and 2.7.

$$\frac{N}{N_0} = 10^{-kH_0} \qquad \text{Equation 2.6}$$

$$\frac{N}{N_0} = 1-(1-10^{-kH_0})^{10^d} \qquad \text{Equation 2.7}$$

where N_o is the number of microorganisms before irradiation, N is the number of surviving microorganisms after irradiation, k (m^2/J) is the slope of the linear part of the survival function, H_0 (J/m^2) is the fluence and d is the intercept of the linear part of the survival function with the ordinate (Cabaj et al., 2002).

UV dose-response is affected by particle-association and clumping of microorganisms. Solids present in wastewater samples can cause a tailing or

flattening of the dose-response curve at higher inactivation levels because clumping or particle association shields a fraction of the microorganisms from the UV light.

2.2 BIODOSIMETRY INDICATOR ORGANISMS

There are usually insufficient numbers of pathogens or surrogate microorganisms naturally present in the source water to allow measurement of inactivation efficiency. In biodosimetry, the reactor influent is often seeded with a suitable indicator organism in sufficient numbers to observe the desired inactivation levels. The ideal surrogate microorganism for evaluation of the performance of UV reactors used for UV microbial reduction in potable water should have the following properties:

- The surrogate should be inexpensive, non-pathogenic and easy to culture in the laboratory in large numbers:
- The surrogate should be of equal or greater resistance than the pathogenic microorganisms of interest:
- The UV dose response of the surrogate should be well-defined and reproducible:
- Surrogates should be reliably detected at low numbers, and
- Surrogates should not replicate in water.

It has been shown that germicidal efficiencies for one species of bacteria or virus can be used to represent the relative response to UV light for all bacteria and viruses (Giese and Darby, 2000). However, correction factors must be applied to account for differences in dose-inactivation relationships. No correction is required for surrogates

with lower UV resistance than the target pathogen, but overly conservative estimates of target pathogen inactivation may result.

Because one of the main tasks of this study is to produce surrogates that break through the filtration process, it is useful to have a microorganism that is smaller in size than the pathogenic microorganisms, C. *parvum*, that could form a cyst or spore but meet the characteristics of a suitable surrogate. Two common surrogates used in drinking water research are *B. subtilis* spores and MS2 coliphage.

2.2.1 *Bacillus subtilis* spores

2.2.1.1 Biology of *B. subtilis*

The genus *Bacillus* is a member of the family Bacillaceae, which are mostly aerobic and facultative bacteria. The characteristics of the family include rob shaped vegetative cells, being gram-positive, and motility by lateral flagella and the ability to form endospores under certain environmental conditions (Maruo and Yoshikawa, 1989). The ability to form resistant spores gives the species an advantage in surviving periods of dryness and nutrient deprivation. The spores have high resistance to chemical degradation, heat, radiation, UV light and dry conditions. *B. subtilis* have a spore coat, which protects the spore from chemical agents and many other environmental stresses including dehydration, cortex, and inner spore membrane surrounding the protoplast and they can remain dormant and viable for very long periods (Prescott et al., 1996). Most research involving drinking water and bacterial spores have used *B. subtilis* (Chang et al., 1985; Qualls et al., 1989; Rice et al., 1996; Sommer and Cabaj, 1993; Sommer et al., 1998; Uvbiama and Craik 2005).

Endospore formation is most efficient when the vegetative cells have grown for at least six or seven generations in a nutrient rich medium before the onset of sporulation (Doi, 1989). The spores can be detected by heating at 70 to 80 °C for 10 minutes and incubating on an appropriate growth medium. Only endospores and some thermophiles will survive this treatment (Prescott et al. 1996).

The benefits of using *B. subtilis* as a surrogate for studying UV disinfection are as follows:

- The spores have high resistance to UV light,
- Spores remain viable for long periods of time,
- They are non pathogenic,
- Spores do not reproduce in samples, and
- Very few other organisms that are present in natural water will return a false positive in the enumeration of viable spores (Prescott et al., 1996).

More importantly, the *Bacillus subtilis* spore (1.2 to 1.5 um) is in the same size range as *C. parvum* oocysts (3 to 5 um) compared to that of viral particles (0.001 to 0.025 um).

2.2.1.2 Dose Response of *B. subtilis* spores

Bacillus subtilis spores have been used in numerous laboratory and pilot scale UV reactor studies to assess UV inactivation against microbes. Most of the UV inactivation curves in the literature exhibit a shouldering curve (Chang et al., 1985; Sommer et al., 1998; Qualls et al., 1983; Qualls et al., 1989; Sommer and Cabaj,

1993; Harris et al., 1987; Mamane-Gravetz and Linden, 2004; Uvbiama and Craik, 2005). Harm (1980) reported that shoulders are usually characteristic of double-stranded DNA. Based on information collected from the literature, the average UV dose at specific inactivation levels was computed.

Numerous studies investigating the inactivation of *B. subtilis* in water show variation in the UV dose necessary for a given log inactivation of *B. subtilis.* Table 2-2 summarizes data from nine studies. As the table shows, the dose required for 2 log inactivation of *B. subtilis* varied from 19 to 62 mJ/cm^2. Each of these studies reported correct procedures for determining the delivered UV dose including accounting for sample absorbance and depth. The factors that may be responsible for this variation in dose include (a) uncertainties in UV dose measurement, calculation, and determination, (b) differences in growth media used to culture the spores, and (c) use of different water matrixes and differences in UV exposure methodology. The dose response of spores to UV radiation is affected by the age, density, sporulation medium, lysozome sensitivity and heterogeneity of the DNA.

According to the mean of these studies, UV doses of approximately 24, 37, 46 and 49 mJ/cm^2 will be able to achieve 1, 2, 3 and 4 log inactivation of *B. subtilis*, respectively.

Table 2-2. Summary of the UV dose required per log reduction of *B. subtilis* in water.

Reference	UV dose / log reduction mJ/cm^2			
	1 log	2 log	3 log	4 log
Qualls et al (1983)	12	19	24	29
Chang et al (1985)	36	49	61	78
Harris et al (1987)	16	22	27	33
Quall et al (1989)	36	62	67	
Sommer and Cabaj (1993)	22	35	40	54
Sommer et al (1998)	28	41	49	
Mamane-Gravetz and Linden (2004)	22	37	47	
Uvbiama and Craik (2005)	17	28	38	49
Mamane-Gravetz and Linden (2006)	25	41	59	
Mean of 9 articles	24	37	46	49
Standard deviation of 9 articles	8	13	14	17

2.2.1.3 UV Inactivation curve modeling for *B. subtilis* spores

The target theory, which is closely related to the second law of photochemistry, is important in understanding the modeling of inactivation curves of spores. The target theory states that a finite number of targets are on every individual cell and that these targets are vulnerable to randomly distributed hits (Harm, 1980). Under the one-hit one-target theory, one hit is assumed to inactivate a cell. As, a result, the inactivation curve should be linear and follow the Chick-Watson law. This result has been

observed to be the case for most single-stranded DNA and RNA in viruses (Harm, 1980). However, in microorganisms with double-stranded DNA and RNA, several targets, n, require one hit (multi-target) or one target requires a series of hits, n, (multi-hit or series events) before the microorganisms are inactivated. Hence, the multi-target and multi-hit models describe the shoulder phenomenon better than one-target one-hit model.

The multi-target and multi-hit models are given by Equations 2.8 and 2.9, respectively.

The multi-target is given by:

$$\frac{N}{N_0} = 1 - (1 - e^{k_m It})^{n_c} \qquad \text{Equation 2.8}$$

and the multi-hit (series event) is given by

$$N = N_0 e^{-k_s It} \sum_{i=0}^{n-1} \frac{(k_s It)^{n_c}}{n!} \qquad \text{Equation 2.9}$$

where N and N_0 are the final and initial number of microorganisms, k_m and k_s are the first-order rate constant (cm^2/mJ) for the multi-target and series event models, I is the irradiance (mJ/cm^2), t is the exposure time(s), and n_c is the number of critical targets for the multi-hit and multi-target model, respectively.

The multi-target and multi-hit models explain the lag phase (shoulder) in *B. subtilis* inactivation curves.

2.2.2 MS2 coliphage

2.2.2.1 Biology of MS2 coliphage

MS2 coliphage are small, icosahedral, non-enveloped, F-specific, single stranded RNA viruses in the Leviviridae morphological group (van Duin, 1988, Prescott et al., 1996). The phage diameter is typically 26.0 to 26.6 nm. The phage can only reproduce in actively metabolizing *E.coli* bacteria. They attach to the F-pili of their host and enter the cell by an unidentified mechanism. Having only three or four genes, genetically it is one of the simplest known phages. Single-standed RNA phage are among the smallest things that carry the necessary information for self-replication and transmission (Van Duin, 1988). One protein is involved in phage adsorption to the host cell and possibly also in virion construction and maturation. The other three genes code for a coat protein, RNA replicase, and a protein needed for cell lysis (Prescott et al., 1996).

The benefits of using MS2 phage as a surrogate include the following:

- It has a high resistance to UV relative to bacterial spores,
- It is non pathogenic to humans,
- The virus does not have photoreactivation capabilities,
- Its UV dose-inactivation relationship is reproducible and follows first order kinetics,
- It has a high resistance to UV relative to bacterial spores, and
- MS2 is easily generated in large numbers up to 10^{12} pfu/mL (Havelaar et al., 1990), whereas *B. subtilis* spores can only be cultured up to roughly 10^{8} pfu/mL (Qualls and Johnson, 1983).

2.2.2.2 Dose Response of MS2 coliphage

The inactivation of MS2 phage has been examined in many bench scale and pilot scale UV reactor experiments. In general, this coliphage has been found to be suitable for use as a biodosimetric indicator for both wastewater and drinking water applications.

Numerous studies investigating the inactivation of MS2 coliphage in water show variation in the UV dose necessary for a given log inactivation of MS2 coliphage. Table 2-3 summarizes data from eight studies. As the table shows, the dose required for 2 log inactivation of MS2 varied from 28.6 to 43.8 mJ/cm^2. Each of these studies reported correct procedures for determining the delivered UV dose including accounting for sample absorbance and depth. The factors responsible for this variation in dose may include uncertainties in UV dose measurement, calculation, and determination; *Escherichia coli* host selection; method employed to culture, purify, and assay MS2 coliphage; use of different water matrixes; differences in experimental UV exposure methodology; or seemingly insignificant disparities in general lab protocol (Batch et al., 2004). As compared between Table 2-2 and Table 2-3, MS2 seems to be somewhat less variable than *B. subtilis* in terms of UV dose-response.

Table 2-3. Summary of the UV dose required per log reduction of MS2 coliphage in water.

Reference	UV dose / log reduction mJ/cm^2			
	1 log	2 log	3 log	4 log
Rauth (1965)	16.9	33.8		
Havelaar et al (1990)	21.9	43.8	65.7	
Wilson et al (1992)	15.9	34	52	71
Nieuwstad and Havelaar (1994)	20.3	40.6	60.9	81.2
Meng and Gerba (1996)	14.0	28.6	44.8	65.2
Tree et al. (1997)	12.1	30.1		
Sommer et al. (2001)	20	38	57	
Nunaualsuwan et al (2002)	17	35	64	97
Mean of 8 articles	17.3	35.5	57.4	78.6
Standard deviation of 8 articles	3.3	5.1	7.9	13.9

2.2.2.3 UV Inactivation curve modeling for MS2 Coliphage

Most studies have shown that the UV dose response-curve against MS2 coliphage is linear, and is consistent with a one-hit or one-target mechanism. Under the one-hit one target theory, one hit, that is the absorbance of a single photon, is assumed to be able to inactivate a cell. As a result, the inactivation curve should be linear and follow the Chick-Watson law. This result has been observed to be the case for most single-stranded DNA and RNA in viruses (Harm, 1980).

A functional representation of a linear was provided in Equation 2.6.

2.3 EFFECT OF PARTICULATE MATTER ON UV INACTICATION OF MICROORGANISMS

Water quality is important when the efficacy of UV disinfection of drinking water is evaluated (USEPA, 1999). Suspended particles in unfiltered water can cause negative effects on the effectiveness of UV light for microbial disinfection because they absorb, scatter, and block UV light (Qualls et al., 1983). Particles can affect the disinfection efficiency of UV by harboring bacteria and other pathogens, partially protecting them from UV radiation, and scattering UV light (USEPA, 1999). Figure 2-4 illustrates the scenario's where light can be blocked from reaching microorganisms attached to the particles. UV light absorbed by particles is no longer available for disinfection. Scattered light is still available for disinfection, but scattering reduces available UV energy by increasing the path length of light traveling through a liquid medium, leading to dissipation of UV energy into the water column (Huber and Frost, 1998). Suspended particles block UV light by shading and shielding microorganisms. Similarly, microorganisms can aggregate or clump together, forming particles that potentially protect pathogens within aggregates that would otherwise be inactivated.

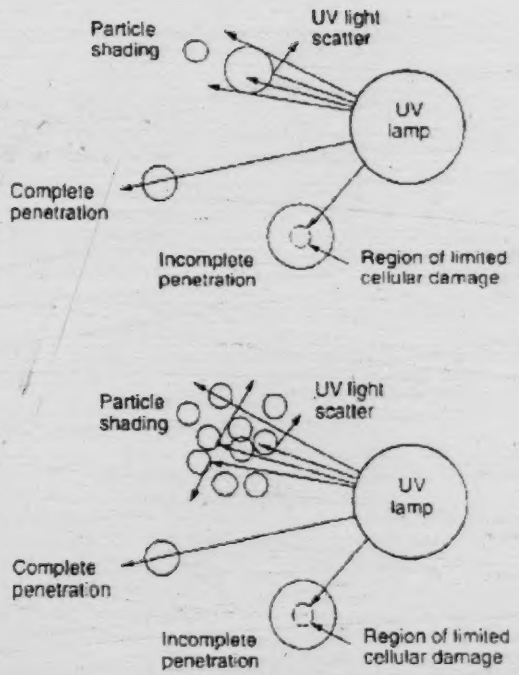

Figure 2-4. Particle Interactions that Impact UV Effectiveness on Microorganisms. (Reproduced from USEPA, 1999)

Post-filtration is the only point of application for UV disinfection in a water treatment process train that is supported by current research (Malley, 2000). After filtration, upstream treatment processes have removed most of the water quality factors that attenuate UV light. The US Environmental Protection Agency's Long Term 2 Surface Water Treatment Rule, which became effective January 2006, specifies that for systems using conventional and direct filtration, the combined filter effluent turbidity must not exceed 1 NTU (USEPA, 2006). For turbidity within filtered quality guidelines and lower, the negative effects of water quality on UV disinfection efficiency have shown to be minimal (Linden et al., 2002). However,

turbidity excursions associated with filter breakthrough could adversely affect UV reactor performance.

Some public water supplies using pristine source waters are exempt from filtration if they meet the filtration avoidance criteria (USEPA, 2002). Turbidity limits for unfiltered surface waters established by the 2006 LT2ESWTR are not to exceed 1 NTU on any day. During times of source water variation, such as during storms, raw water turbidity may reach levels beyond 5 NTU. From 1 to 10 NTU and beyond, the effects of suspended particles on UV dose delivery are not well-understood. Research is needed to demonstrate the effectiveness of UV disinfection at these levels of turbidity.

Previous research has shown that suspended particles in wastewater can increase microbial survival by increasing UV absorbance of the water and by providing a substrate for microbial growth, which physically shields microorganisms from UV light (Emerick et al., 1999; Loge et al., 1999; Parker and Darby, 1995; Harris et al., 1987). It has been reported that wastewater particles 7 to 10 um in diameter and larger are capable of shielding particle-associated organisms (total coliform bacteria) from UV light (Jolis et al., 2001; Emerick et al., 2000; Qualls and Johnson, 1985; Qualls et al., 1983). In addition, fecal coliform survival is directly proportional to suspended solids concentration for secondary wastewater effluents (Tchobanoglous et al., 1999; Whitby and Palmateer, 1993), and UV absorbance characteristics of wastewater particles vary among different treatment effluents (Loge et al., 1999). Although research conducted on wastewater provides an indication of the UV attenuation caused by particles in drinking water supplies, characteristic variations

that exist between surface water and wastewater particles are likely to have different effects on the transmission of UV light through a turbid water column.

To examine the effects of suspended particles on UV disinfection of drinking water, one study evaluated the effects of turbidity in three water samples (filtered drinking water, settled alum floc, and settled wastewater solids) on the applied UV dose required for 2-log inactivation of MS2 coliphage (Malley, 2000). Results of bench-scale irradiation experiments showed that for filtered drinking water, an increase from 1 to 5 NTU required a UV dose increase of approximately 50% to achieve 2-log MS2 coliphage inactivation. The UV dose increase necessary to accomplish 2-log inactivation at increasing turbidity was likely a result of two factors: particle association and shielding of microorganisms from UV light and effects of particles on the transmission of UV energy through the water column.

Jolis et al. (2001) conducted pilot-scale and bench-scale coliform inactivation tests with UV light to show how suspended solids remaining in filtered secondary effluent affect the efficiency of the UV disinfection process. Inactivation rates decreased with increasing concentrations of suspended particles with size > 7 um in tertiary effluent. It was concluded that the efficiency of the UV disinfection process is largely dependent on the amount of suspended particles in the tested water capable of shielding coliform bacteria from radiation.

Wright et al. (2002) explained that for a given wastewater, tailing is a function of the concentration of the wastewater particles > 8 um in size. Tailing may also be a function of the percentage of particles that contain the target microbe. For example, for the activated sludge process, the percentage of particles containing total coliform

bacteria had a negative correlation with solids retention time (SRT). For a given UV dose, better disinfection was observed with activated sludge processes with long SRT.

Amoah et al. (2005) found that an increase in the concentration of biological and non-biological particles within 5 to 25 um size range that were concentrated from the bottom sediment of Lake Okanagan, British Columbia, Canada led to a statistically significant but modest reduction in the inactivation of samples seeded with *C. paruvum* oocysts and *G. muris* cysts as turbidity increased from 0.3 to 20 NTU and 7.5 to 20 NTU, respectively.

Mamane-Gravetz and Linden (2005) analyzed lake water samples for the presence of indigenous aerobic bacterial spores and their response to UV disinfection. Tailing of the UV inactivation curves resulted from aggregation of a portion of the spore population due to hydrophobic interactions. The isoelectric point for optimum attachment of isolated spores was found to be reached at acidic pH values that are not typical of natural or treated waters.

Mamane-Gravetz and Linden (2006) found that aggregation of microbe with particles can reduce the effectiveness of UV disinfection. This experiment evaluated the comparative impact of dispersed spores mixed with clay particles, spore-spore aggregates and spore-clay aggregates on UV disinfection performance in simulated drinking water. They concluded that:

- particle parameters such as particle concentration, volume and surface area per milliliter are reliable indicators for removal of *B. subtilis* spores,
- pH does not affect UV inactivation of *B. subtilis* spores spiked into a simulated water matrix,

- aggregate composition is non-homogeneous with respect to the number of *B. subtilis* spores and clay particles within aggregates and with respect to the ratio of spores and clay particles among aggregates in the spore-clay system,
- approximately 30-50% of the *B. subtilis* spores in aggregates are protected from UV irradiation, but the percent of spores within an aggregate protected from UV decreases with increase in fluence.

However, some studies on particle-association using filtered and unfiltered water have produced different results. For example, Passantino et al. (2004) and Batch et al. (2004) also found that the presence of particles did not have a significant effect on the UV inactivation of microorganisms. Passantino et al. (2004) compared the UV dose-response of bacteriophage MS2 seeded in waters with montmorillonite clay turbidity up to 12 NTU and algal content up to 42 ug/L (as chlorophyll A) to the UV dose-response of phage MS2 seeded into de-ionized water. Also considered was the inactivation of bacteriophage MS2 in the presence of naturally occurring turbidity in reservoir waters. UV light was effective for inactivating MS2 phage in the presence of montmorillonite clay, algae, and naturally occurring turbidity in collimated-beam testing. The levels of turbidity and algal cells typically present in unfiltered drinking water supplies did not negatively affect the ability of UV light to effectively inactivate seeded phage. One possible explanation for this is that the turbidity and algal levels tested were relatively low and there were simply not enough particles to interfere with the UV light. Another possibility is that the phage did not associate with the particles, leaving the phage susceptible to UV disinfection. Passantino et al.

(2004) noted that because of the low concentration of microorganisms present in drinking water sources, it is not practical to study the naturally occurring interactions between particles and microorganisms.

Batch et al. (2004) reported that UV radiation was effective for inactivating MS2 coliphage seeded into filtered drinking water that met federal regulations when UV dose measurements took turbidity, particle count and absorbance into consideration.

2.4 NEED FOR THIS RESEARCH

Ultraviolet (UV) radiation is rapidly becoming the technology of choice for providing additional protection against microorganisms in drinking water treatment plants in Alberta. However, until now, most of the UV dose-response studies of microorganisms have been based on relatively clean water. The UV dose table established by USEPA in the LT2SWTR (USEPA, 2006) for determination of *C. parvum* and *G. lamblia* inactivation credit in operating UV reactors was developed based on studies where the microorganisms were spiked into laboratory water during UV exposure. Particle-association of microorganisms needs to be accounted for in UV reactors design and validation because studies have shown that UV disinfection is affected by the presence of particles in water, especially if the microorganisms are completely engulfed by the particles or floc material. In a multi barrier water treatment plant, microorganisms have to pass through several water treatment processes prior to UV disinfection. These upstream processes typically consist of coagulation, flocculation, settling and filtration. These processes might promote the aggregation of the microorganisms and the particle association. The possible result is

that the UV radiation reaching the target microorganisms might be reduced or completely blocked. Therefore, the dose response curve generated for pure microorganisms might not be able to estimate the UV dose required for attaining a certain level of inactivation in filtered water.

Few studies have attempted to simulate the upstream water treatment processes in order to observe UV disinfection in aggregation of the microorganisms and the particle association. The floc particles present in poorly filtered water may be able to completely engulf the microorganisms and prevent UV light penetration. Templeton et al. (2005) studied whether colloid-sized particles can enmesh and protect viruses from ultraviolet light and sought to determine the particle characteristics that are most relevant in causing a protective effect. A synthetic mixture of water containing three different types of particles (Kaolin clay, humic acid powder and activated sludge) were spiked with the viral surrogates and coagulated at optimum condition. Then the coagulated water was exposed to UV at different dose levels. The results of this study suggested that particles less than 2 μm in diameter are large enough to protect viruses (MS2 coliphage and bacteriophage T4) from UV light and that particulate chemical composition may be a critical factor in the survival of particle-associated viruses during UV disinfection. The limitation of the Templeton et al. (2005) study is that the sample treatments were not representative of actual water treatment plant conditions, although the study did shed some light on the effect of coagulation and flocculation on UV inactivation of the microorganisms. Because the flocs created were relatively weak and feathery, they might not have prevented the penetration of UV light as effectively as flocs in an actual water treatment process.

Uvbiama and Craik (2005) examined the potential effects of upstream treatment processes and the state of aggregation on the UV inactivation rates of microorganisms present in filtered and backwash water. This research used a homogenizing step for breaking up the aggregated spores either before or after UV exposure. The results of this research showed the UV inactivation rate of *B. subtilis* spores present in the filtered water and filtered backwash water was significantly different at the 95% confidence level from that of spores suspended in buffered de-ionized water. These differences decreased when samples were homogenized before UV exposure. This study showed that the apparent UV resistance of microorganisms present in filtered and backwash water might be greater than the UV resistance of mono-dispersed microorganisms due to aggregation.

Using a similar approach to that of Uvbiama and Craik (2005), Mahmud (2006) also examined the potential effects of upstream treatment processes and the state of aggregation on the UV inactivation rates of microorganisms present in filtered water. However, the author used the pathogen, *C. parvum,* in his experiments instead of the surrogate *B. subtilis* used by Uvbiama and Craik (2005). The results of this research showed the UV inactivation of *C. parvum* oocysts was reduced by 0.8 and 1.1 log-unit at 5 and 40 mJ/cm^2 respectively as a result of aggregation. These results suggested that the UV inactivation of *C. parvum* oocysts in poorly filtered water might be overestimated if the effect of oocyst aggregation is not taken into consideration. Moreover, the Uvbiama and Craik (2005) and Mahmud (2006) studies were done using a relatively limited set of conditions (using just one source water, North Saskatchewan River), a single alum dosing condition, and no information was

generated on viruses. Since viruses are much smaller than oocysts of *C. parvum*, the effect of aggregation on UV might be very different.

The research study described in this thesis is similar to the study conducted by Uvbiama and Craik (2005) and Mahmud (2006) except that two microorganisms (*B. subtilis* spores and MS2 coliphage) were used as a target microorganism because they act as surrogates for pathogenic microorganisms and are relatively easy and inexpensive to produce and to enumerate. In order to determine if the extent of aggregation and the effect on UV inactivation can be predicted based on readily measurable filtration performance variables, different coagulant doses were used to simulate different levels of turbidity and particle concentration in the filtered water, using different water sources collected during different seasons (stable winter conditions versus spring run-off). Consequently, this study will help establish engineering safety factors for UV systems that would guarantee the expected level of disinfection performance in filtered water and ensure protection of public health.

3. EXPERIMENTAL MATERIALS AND METHODS

This chapter details the experimental materials and methods used in this study. This chapter is divided into raw water sample collection procedures, water treatment process simulation, UV exposure methods, *B. subtilis* spores methods and MS2 coliphage methods. The experiments in this thesis were conducted in five different condition waters. Preliminary experiments were conducted in September 2006 using North Saskatchewan River (NSR). Mid-winter experiments were conducted in February 2007 using North Saskatchewan River (NSR) and in March 2007 using Bow River (BR). Spring run-off experiments were conducted in March 2007 using Red Deer River (RDR) and in April 2007 using North Saskatchewan River (NSR).

3.1 RAW WATER SAMPLE COLLECTION

The experiments in this thesis were conducted from three kinds of river water from three sources in Alberta; the North Saskatchewan River, the Bow River and the Red Deer River. North Saskatchewan River water samples were collected in 20 L plastic containers from the Rossdale water treatment plant quality control laboratory in Edmonton, AB. Bow River and Red Deer River were collected at the City of Calgary Bearspaw water treatment plant and the City of Red Deer water treatment plant, respectively, and were shipped to University of Alberta by overnight courier. Prior to the collection, the containers were soaked into 30% bleach overnight and washed thoroughly with deionized water at least 3 times. Bow Reservoir and Red Deer River were shipped from Calgary and Red Deer. After collecting, the raw water samples were stored at 4°C until their use in the experiments. Table 3-1 shows the water quality characteristics of the raw water samples.

Table 3-1. Water quality characteristics of the raw water samples. Data provided by EPCOR Water Services.

Water parameter	North Saskatchewan (Sep, 2006)[a]	North Saskatchewan (Feb, 2007)[a]	North Saskatchewan (Apr, 2007)[b]	Bow River (Mar, 2007)[a]	Red Deer River (Mar, 2007)[b]
Turbidity, NTU	2.2	4.24	523	2.93	164
pH	8.49	8.03	7.95	8.33	7.73
Conductivity, us/cm	330	360	301	333	312
Total Hardness	164	182	137	173	126
Alkalinity, mg/L	125	131	119	128	148
Color, TCU	4.2	3.9	43.4	4.2	126.2

[a] samples represented stable winter conditions.
[b] samples represented spring run-off conditions.

3.2 WATER TREATMENT PROCESS SIMULATION

3.2.1 Coagulation, flocculation and settling

A jar testing apparatus (PB-700 Jar tester, Phipps and Bird, Richmond, VA) was used to simulate the coagulation, flocculation and settling process at bench scale (Figure 3-1). The jar testing protocol used in this study was similar to that used by EPCOR Water Services at the Rossdale water treatment plant. One jar (2L B-kars) was filled with 2L of raw river water. This jar was seeded with *B. subtilis* or MS2 coliphage to approximately 10^6 CFU/mL and 10^6 PFU/mL, respectively. The contents were then mixed at ~ 75 rpm for about 30min. Mixing was increased to 300 rpm, and an alum(Cleartech, Edmonton, AB) was added simultaneously. This alum dose was

optimal, sub-optimal and poor as determined for the test water in preliminary jar tests. After 60 s of rapid mixing, the speed was reduced to 75 rpm, and Percol LT27A anionic polymer(Allied Colloids Inc., Brampton, ON) was added immediately. After 3 min, the speed was reduced to 50 rpm for another 3 min, and finally to 25 rpm for 3 min. The water was allowed to settle for an additional 3 min. The settled water from the jar was transferred to a sterile flask, which was connected to a laboratory-scale dual media filter column. The average turbidity of the settled water was measured on a Model 965 Turbidimeter (Orbeco Hellige Turbidimeters, Orbeco Analytical Systems, Farmingdale, NY).

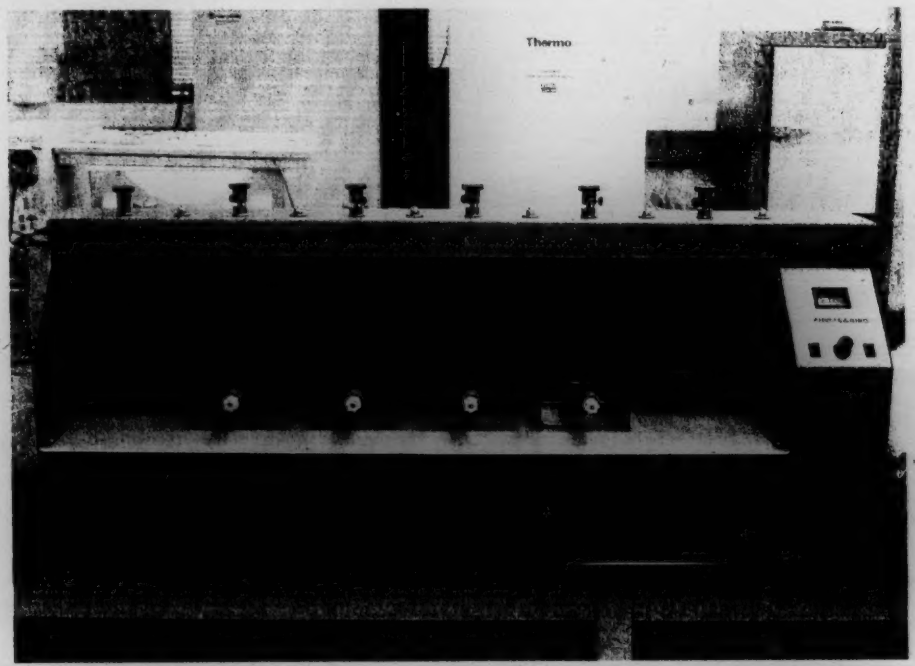

Figure 3-1. Jar testing apparatus used for simulation of coagulation, flocculation and settling

3.2.2 Filtration

The filtration procedure used in this study was adopted from Mahmud (2006). Mahmud (2006) used a bench-scale 2.5 cm ID filter column. Because the primary purpose of the experiment was to simulate the aggregation of microorganisms that may be encountered in conventional drinking water treatment systems and was not to assess the performance of the filter, the use of a relatively small diameter column was considered acceptable. The filter column consisted of a 100 cm long tube (2.54 cm internal diameter) and an overhead tank (10.16 cm internal diameter) constructed of clear PVC material. The filter was designed and operated in a constant head declining rate mode. The 100 cm long clear PVC pipe was filled with 20 cm and 7.2 cm of crushed quartzite filter sand and anthracite filter grade coal, respectively (AWI Filters, Calgary AB), as shown in Figure 3-2.

Table 3-3 provides the physical properties of the sand and anthracite coal. Prior to filtration, the filter column was backwashed for about an hour to remove any silt and dust from the media. The settled water from the jar test procedure was pumped at a flow velocity of 18.8 m/h from the gently stirred reservoir to the inlet of the filter head tank using a peristaltic pump. A peristaltic pump was selected for this purpose to avoid breaking up the floc formed during coagulation and flocculation. A constant-head was maintained on the filter by virtue of the head-tank and overflow. Overflow flow velocity was maintained at 12.8 m/h. Flow velocity through the filter at the beginning of the filtration run was 4.9 m/h. The filtered water generated during the filtration run was collected as a composite sample. Sub-samples were also collected at various times during the filtration run, and the turbidity, absorbance and spore

concentration were measured. Table 3-2 shows each of the alum and polymer doses combinations used in this thesis and the resulting turbidity of the water after filtration.

Table 3-2. The dose of alum and polymer used for coagulation in different raw water samples and after filtering NTU of the raw water samples by jar test.

	Alum, mg/L			Polymer. mg/L
	Optimal	Sub-Optimal	Poor	
North Saskatchewan (Sep, 2006)[a]	20 (0.22NTU)	9 (0.96 NTU)	5 (1.81 NTU)	0.33
North Saskatchewan (Feb, 2007)[a]	25 (0.25 NTU)	10 (1.15 NTU)	7 (1.92 NTU)	0.5
Bow Reservoir (Mar, 2007)[a]	25 (0.28 NTU)	9 (1.18 NTU)	5 (2.42 NTU)	0.5
Red Deer River (Mar, 2007)[b]	100 (0.30 NTU)	75 (0.94 NTU)	50 (2.35 NTU)	1
North Saskatchewan (Apr, 2007)[b]	100 (0.24 NTU)	50 (1.12 NTU)	40 (2.48 NTU)	1.2

[a] samples represented stable winter conditions.
[b] samples represented spring run-off.

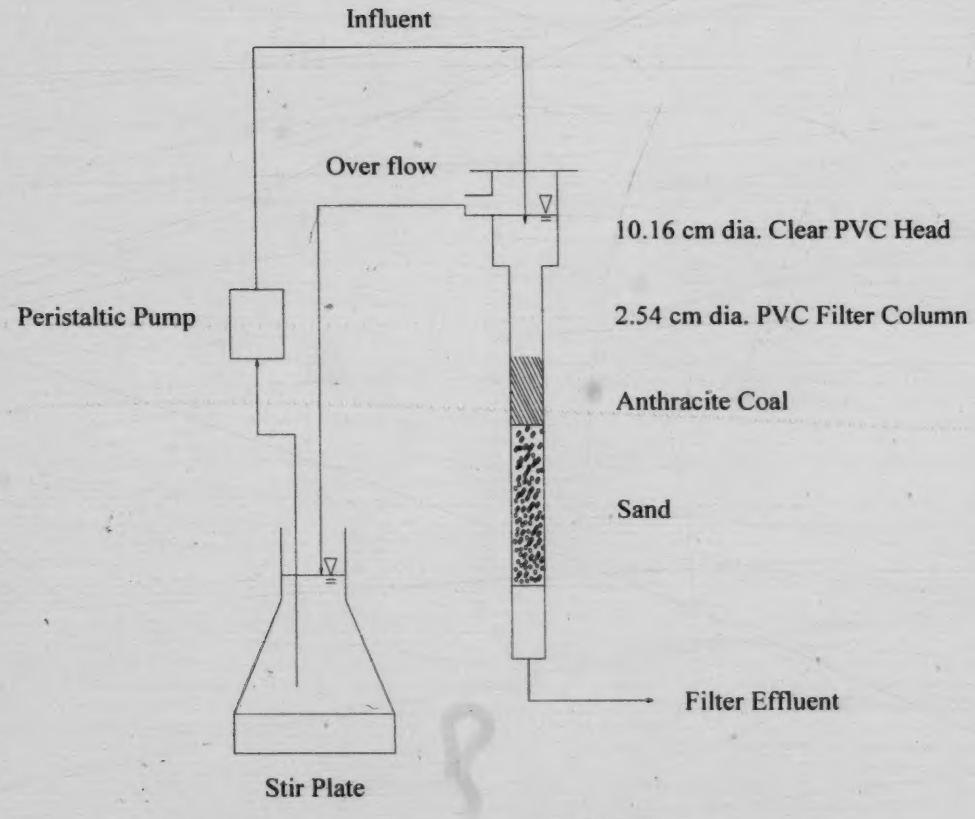

Figure 3-2. Schematic diagram of the simulated filtration process

Table 3-3. Physical properties of sand and anthracite coal (AWI Filter)

Property	Sand	Anthracite Coal
Bulk density, kg/m^3	1362(dry basis)	N/A
Sphericity	< 0.6(highly angular)	N/A
Moh hardness	7	N/A
Acid solubility	Nil	N/A
Specific gravity	2.60 - 2.65	1.4 - 1.6
Effective size	0.35mm	0.8-0.9 mm
Uniformity coefficient	< 1.5	N/A

N/A – Not available

3.3 UV Exposure Methods

3.3.1 Collimated beam apparatus

UV exposure experiments were carried out using a collimated beam apparatus (Calgon Carbon Corporation, USA). A 10 W low pressure mercury arc lamp (Ster-L-Ray Germicidal Lamp, model G12T6L, 15114, Atlantic Ultraviolet Corporation, Hawpange, NY) housed in the collimated beam unit was used for all the UV exposure trials. The unit uses a pneumatically driven shutter which can be opened and closed to allow the UV light to irradiate the test suspension under controlled environment. The time required for delivering the required UV dose was keyed in to a timing device which automatically controlled the opening and closing of the shutter. A collimation tube (24 cm long) placed beneath the lamp was used to make sure that the UV rays reaching the test suspension were reasonably parallel and uniform. The distance from the lamp to the surface of the test water sample for all the UV exposure trials was 45.5 cm. The test water sample was placed on an adjustable platform mounted over a stirrer (Figure 3-3).

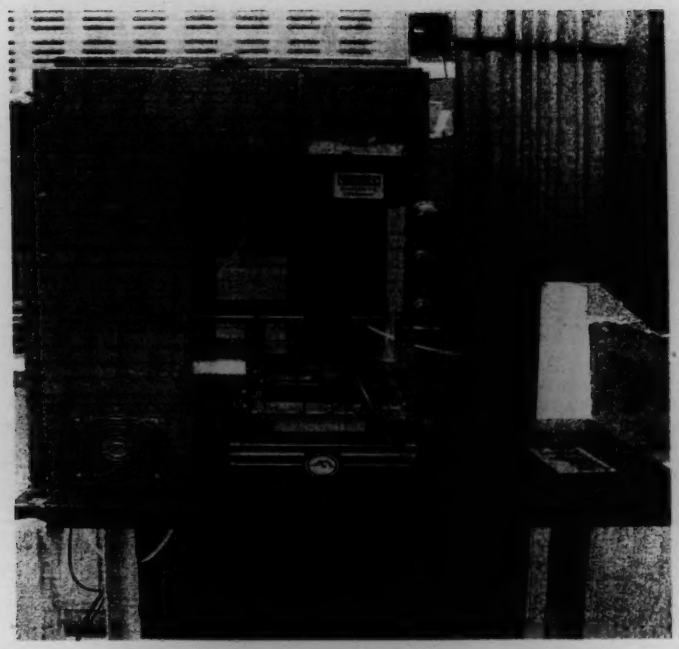

Figure 3-3 Collimated beam apparatus used for UV exposure experiments

3.3.2 Homogenization

The objective of the homogenization step was to break up aggregates and disperse *B. subtilis* spores attached to or embedded within aggregates. Inactivation was also compared to that of spore preparations suspended in clean, particle-free de-ionized (DI) water. The PowerGen 700 (Model 700, Fisher Scientific, Pittsburgh, PA) is a variable speed (10 000 to 30 000 rpm) post-mounted homogenizer that is normally used for breaking apart tissues into individual cells (Figure 3-4). The external diameter of the homogenizer shaft is 7 mm. The homogenizer shaft is protected by the generator tube. A knife (6 mm diameter and 12.7 mm long) is attached to one end of the shaft. The rotation of the shaft and knife at high speed in liquid performs the homogenizing operation. The particles and clumps were probably broken down by the shearing action created by the high-speed bubbles emanating from the perforated

end of the generator tube housing the knife. For samples homogenized before and after UV exposure, the 10 mL samples in the test tubes were homogenized at 30 000 rpm for 30 s using a 20 mL beaker.

Figure 3-4 Tissue homogenizer for *B. subtilis*.

3.3.3 Blender

The objective of the blender step was to break up aggregates and disperse MS2 coliphage particles attached to or embedded within aggregates. A phage elution method that involved addition of the elution solution followed by vigorous mixing used to release phage from particles for enumeration. The method was adapted from those previously described by Parker and Darby (1995), Chauret et al. (1999) and Templeton et al. (2005) and involved the addition of a 2%(w/v) beef extract solution

(Sigma, B4888, adjusted to pH 9.0 using NaOH) and blending at 20,000 rpm for three minutes. The Blender 7011 (Model 31BL92, Waring Products, New Hartford) is a variable speed (18 000 to 22 000 rpm) blender(Figure 3-5).

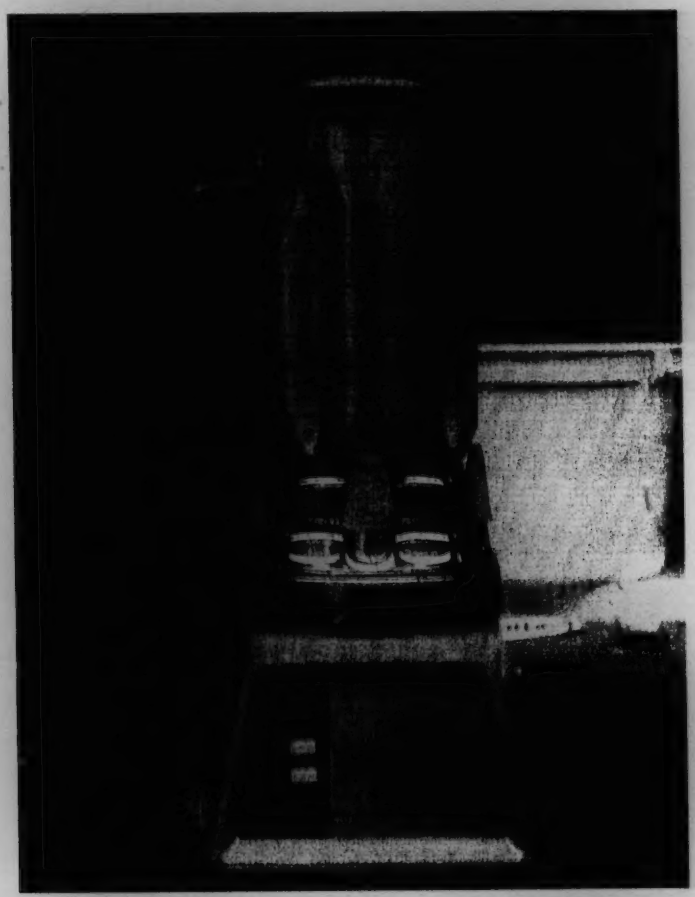

Figure 3-5 Blender used for elution of MS2 Coliphage from aggregates.

3.3.4 Integrating Sphere Spectroscopy

The objective of the Integrating Sphere (IS) spectroscopy is to measure true absorbance in a particulate suspension by accounting for scattering of incident UV light by particles. Conventional absorbance measurements, termed "direct" were performed with a UV-vis dual spectrophotometer. Integrating Sphere (IS)

spectroscopy was performed with the same spectrophotometer equipped with 150mm diameter IS attachment (Shimadzu UV-2200 Series 206-61600) and a center mount sample holder used to position the sample inside the IS, with a reflectance factor of 0.973 at 254nm. The turbid sample was placed in a 1 cm path length quartz cuvette with all four windows optically polished. The cuvette is fixed to a spring loaded holder that is hanged in the center of the sphere, connected to the top sphere cover. See Figure 3-6.

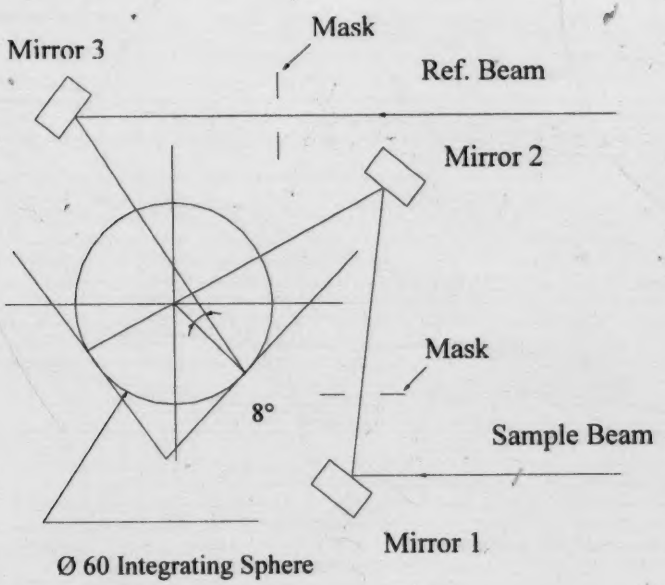

Figure 3-6. Optical system diagram for an Integrating Sphere.

3.4 *B. SUBTILIS* SPORES METHODS

3.4.1 Production

Stock suspensions of *B. subtilis* (ATCC 6633, American Type Culture Collection, Manassas, VA.) spores were produced by using the modified Schaeffer medium method (Leighton and Doi, 1971) with some modifications. Initially, the pre-culture was prepared from freeze-dried spores added to 8 g nutrient broth/L (BBL Nutrient Broth, Benton Dickinson Microbiology Systems, Cockeysville MD). The broth was incubated at 37°C on an incubator shaker (Innova 4080, New Brunswick Instruments Co. Inc., Edison, NJ) at 180 rpm for 24 h. Subsequently, pre-cultures for other batches were generated from isolated *B. subtilis* colonies from streak plates. The growth medium was prepared by adding 0.1 mL/100 mL of a filter-sterilized (0.22 µm Millex® GS Filter unit, Millipore, Cork Ireland) solution of 1 µM $FeSO_4$, 10 µM $MnCl_2$, and 1 mM $CaCl_2$ to sterile nutrient broth (sterilized at 121°C for 15 min). After gentle swirling, the mixture was inoculated with the *B. subtilis* pre-culture and incubated at 37°C on an incubator shaker at 180 rpm for 72 h. On completing sporulation, *B. subtilis* spores were harvested by centrifugation at 7500 × g for 20 min at 4°C. The supernatant was decanted and the pellet re-suspended in sterile DI water. This wash step was done three times. Once complete, spores were pasteurized in a water bath at 80°C for 30 min with intermittent swirling of the solution. The stock solution was finally re-suspended in 50% ethanol, homogenized at 30 000 rpm for 3 min, and stored at 4°C. The concentration of spores in the stock solutions used for this study was ~ 1.5×10^9 CFU/mL. The presence of spores in the stock solution was verified by using Schaeffer – Fulton staining and phase-contrast microscopic

examination.

3.4.2 Spore Enumeration

Spores in the stock preparations and experimental samples were enumerated by using a pour plate technique. A decimal dilution series of the stock or sample was prepared. An aliquot (1 mL) of stock or diluted sample was added to 9 mL of sterile DI water in a 15 mL test tube, and the suspension was vortex mixed (Genie 2 Fisher Vortex, Fisher Scientific, Bohemia, NY) for about 30 s to 1 min at a medium setting. One milliliter of this suspension was transferred aseptically to a sterile 150 x 15 mm sterile polystyrene Petri dish (Fisherbrand). Approximately 15 mL of nutrient agar made from 8 g nutrient broth/L and 16 g solidifying agar/L (Difco Laboratories Detroit, MI) was poured onto the 1 mL suspension. The contents of the dish were gently but thoroughly mixed by using backand- forth and swirling motions. Solidified agar plates were incubated at 37°C for 48 h. The colony forming units (CFU) was counted with the aid of a colony counter (Quebec Colony Counter, Buffalo NY). All plating and enumerations were done in triplicate.

3.5 MS2 COLIPHAGE METHODS

3.5.1 Coliphage Production

Double-layer agar technique was applied in the culture of the MS2 coliphage. MS2 phage strain 15597-B was purchased from the American Type Culture Collection (ATCC, Manassas, VA). An actively growing broth culture of the *E. coli* ATCC 15597 host culture (20 uL of *E.coli* in 10 mL Tryptocase soy broth(TSB,

Difco 0370-17-3)) was prepared 18 to 24 hours before propagating the coliphage. ATCC 271 broth was prepared from 1 L of distilled water to Tryptone (Difco 0123) 10 g, Yeast extract 1 g, and NaCl 8 g. Approximately 1.0 mL of ATCC 271 broth was added to a freeze dried phage vial. Approximately 1.0 ml of ATCC 271 broth is added to a freeze dried phage vial. The bottom agar was prepared as follows. The bottom agar was prepared using 30 g of TSB and 15g of agar in 1000 mL of distilled water, and autoclaved at 121 °C for 30 minutes. The bottom agar was cooled in a water bath at 47 °C, and aseptically poured (approximately 15 – 20 mL) into 100mm Petri plates. Agar was allowed to solidify and plates were stored inverted. The bottom agar plates were overlaid with 2.2ml of a top agar/MS2 phage broth mixture. The top agar was the same composition as the bottom agar, but contained 5 g/L instead of 15 g/L of granulated agar. A molten agar mixture was prepared and was maintained at 43 to 45 °C until plates that were ready to be poured. Approximately 10 drops of *E. coli* host broth was added to every liter of molten top agar. At a ratio of 10 to 1, top agar was added to the broth suspension containing MS2 phage, mixed and spread over the hardened surface of the bottom agar. Plates are incubated upside down for 18 to 24 hours at 37 °C.

To harvest the MS2 phage from the plates, approximately 2 ml of phosphate buffered saline (PBS) solution was added to each plate and then the top agar was harvested from the plate surface. The PBS solution was prepared as follows. The PBS solution was prepared using 32 g of NaCl, 4.6 g of Na_2HPO_4, 0.8 g of KCl and 0.8 g of KH_2PO_4 in 4000 mL of distilled water. The PBS solution with top agar was centrifuged at 1000 rpm for 25 minutes by a refrigerated super speed centrifuge (RC-

5B, Thermo Fisher Scientific, Waltham, MA) and the supernatant was passed through a 0.22 um filter (Supor-200, Gelman Sciences, NY). The phage suspension produced was a mother stock and was stored at 4 °C. MS2 phage stock used in biodosimetry testing was produced using the same procedure as stated above, but using the mother stock as starting material.

3.5.2 *Escherichia Coli* Production

Escherichia coli strain 15597 was purchased from the American Type Culture Collection (ATCC, Manassas, VA). These bacteria are used as the host for generating and enumerating MS2 coliphage. Approximately 1.0 mL of ATCC 271 broth was added to the dehydrated pellet. The rehydrated pellet was then transferred into a tube containing 5 to 6 ml of the broth medium and was vortex mixed (Genie 2 Fisher Vortex, Fisher Scientific, Bohemia, NY). The suspension was then incubated at 37 °C on a shaker table for 24 hours. Subsequent *E. coli* suspensions were prepared by adding one drop of previous cultured *E. coli* broth to a test tube containing 10 mL of ATCC 271 broth. The tube was mixed, split between three test tubes and incubated in the same manner as stated above.

3.5.3 Coliphage Enumeration

MS2 phage was enumerated in a procedure nearly identical to the method used in its production. Refrigerated 100mm diameter plates with hardened bottom agar were warmed to room temperature and 2.2 mL of top agar/MS2 phage broth suspension was poured on top. This mixture was prepared in the same manner described in the

production procedure. The plates were incubated upside down at 37 °C for 18 hours. Plaques formed on the *E.coli* lawn were counted as one viable MS2 phage.

Dilutions of water samples containing MS2 phage were prepared by adding 100 uL of sample to a tube containing 900 uL of ATCC 271 broth. The suspension is mixed and the procedure repeated until the desired phage concentrations were achieved.

3.5.4 Phage Elution Method

A phage elution method was used to release phage from particles for enumeration. The beef extract solution was prepared by adding 25.5 g of beef extract powder (Sigma Aldrich, St. Louis, MO) to 1 L of distilled water and adjusting the pH to 9.0 using 2-3 mL of 50% NaOH solution. For each water sample, 55 mL of this beef extract solution were added to the blender compartment, followed by 15 mL of the sample, for a total volume of 70 mL. Therefore, the phage concentrations in all samples put through this extraction method were multiplied by a dilution factor of 70/15, and blended at 20,000 rpm for three minutes. The liquid was then withdrawn from the base of the compartment using a wide-bore sterile pipette. Tipping was necessary because the blending formed a foam on top of the liquid.

4. ANALYSIS OF RESULTS

This chapter presents the experimental results obtained in the study. The chapter is subdivided into three parts: (1) control experiments, (2) impact of upstream treatment processes on UV inactivation of *B. subtilis* spores, and (3) impact of upstream treatment processes on UV inactivation of MS2 coliphage.

4.1 CONTROL EXPERIMENTS

Experimental controls were necessary to ensure that sample processing steps, such as pasteurization, homogenization and blending, and unknown variables did not have a significant impact on the experimental results. The absence of growth on the negative controls suggests that no contamination of enumerated plates occurred with either *B. subtilis* or MS2 coliphage.

4.1.1 *B. subtilis*

The experiments were carried out with prepared *B. subtilis* spores suspended in DI water. The absorption coefficient of DI water ranged from 0.033 to 0.046 cm^{-1} after the addition of the spores suspension. These experiments were done with a spores from a single batch preparation (Batch 1 spores).

Because *B. subtilis* spores were suspended in the water, contamination by vegetative bacteria present in the water had to be minimized. This procedure was done by heating samples to 75°C after they had been exposed to UV radiation and prior to enumeration. In order to determine the effect of this pasteurization step on the measured UV inactivation of the spores, an experiment was conducted in which

spores in sterile DI water were exposed to several UV doses, with and without pasteurization after UV exposure. With respect to homogenization, the objective of these experiments was to determine whether the homogenization had a significant impact on *B. subtilis* spore aggregation and viability.

Table 4-1 describes the UV irradiation conditions for spores in DI water. The results of these experiments are shown in Figure 4.1. Detailed results from UV exposure trials in DI water are provided in Table A-1 in Appendix A. It is evident that a low level of log inactivation could be achieved even at high UV doses. At 60 mJ/cm^2, the average log inactivation for experiments number 1 to 3 and experiments number 4 to 6 was 2.3 and 3.0, respectively. These values are much lower than those (average 4.0 log inactivation) reported in the literature (Chang et al., 1985; Sommer et al., 1998; Quall et al., 1983; Quall et al., 1989; Sommer and Cabaj, 1993; Mamane-Gravetz and Linden, 2004; Uvbiama and Craik, 2005).

Table 4-1. Description of experiment condition for spores in DI water

Experiment Number	Condition
1	Non-pasteurized and Non-homogenized
2	Non-pasteurized and Non-homogenized
3	Non-pasteurized and Non-homogenized
4	Homogenized and Pasteurized at 75°C for 15 min prior to enumeration
5	Homogenized and Pasteurized at 75°C for 15 min prior to enumeration
6	Homogenized and Pasteurized at 75°C for 15 min prior to enumeration

A two-factor Analysis of Variance (ANOVA) test conducted on the two conditions indicated that there was no statistical different at a 95% confidence level

(p-value = 0.892, n = 30). The non-linear regression fit of the multi-target model (Equation 2.8) to inactivation data is shown in Figure 4-1. Multi-target model parameters determined by non-linear least squares regression for the inactivation of spores in DI water are provided in Table 4-2. The Microsoft® Excel® tool was used to minimize the sum of squares of the predicted and experimental values to obtain model parameters.

Table 4-2 Least-Squares multi-target parameters, number of target, n_c and rate constant, k_m of multi-target model fitted to UV inactivation curves *B. subtilis* spores in DI water (Experiments done with Batch 1 spores).

Sample Condition	Number of targets, n_c	Rate constants, k_m
Non-pasteurized and Non-homogenized	3	0.11
Homogenized and Pasteurized at 75°C for 15 min prior to enumeration	3	0.13

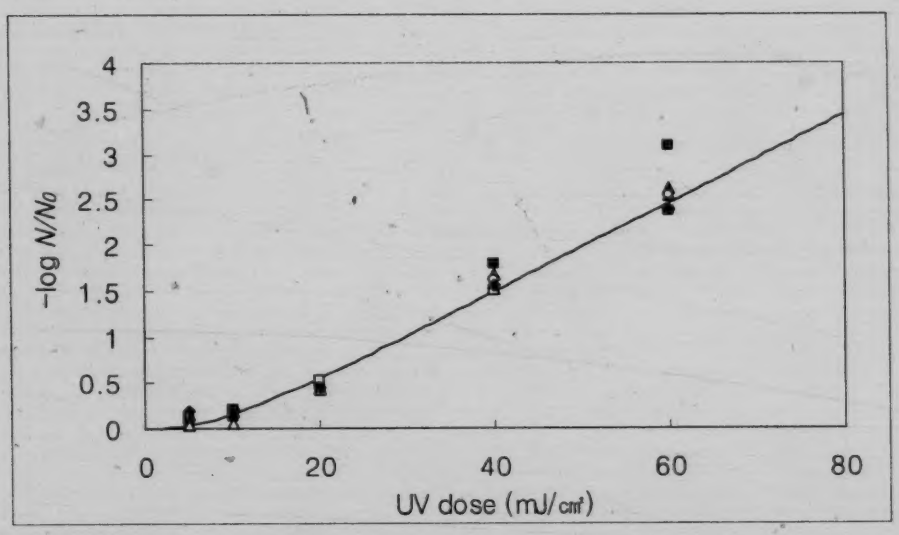

Figure 4-1. A multi-target model predicted *B. subtilis* inactivation vs measured *B. subtilis* inactivation for spores suspended in DI water. Multi-target model (line), experiment Number 1 (■), experiment Number 2 (▲), experiment Number 3 (□), experiment Number 4 (△), experiment Number 5 (○), and experiment Number 6 (●) as described in Table 4-1. Experiments done with Batch 1 spores.

A two-factor ANOVA test indicated that there was no statistical difference between the two treatment conditions replicated three times at a 95% confidence level. However, there is a considerable difference between the computed first-order rate constant values, k_m, determined in this study and those reported in the literature, so in this study checked a second batch preparation of *B. subtilis* spores was tested. Table 4-3 shows the difference in log reduction of the spores used in this study and those reported in the literature.

Results from UV exposure trials in DI water using the second batch preparation *B.subtilis* are provided in Table A-2 in Appendix A. Based on the control experiments in this study results, there is no bias from pasteurization and homogenization.

Table 4-3. Comparison between UV inactivation determined for the spores used in this study to that reported in the literature review for log reduction for *B.sutilis*

Log Inactivation - log N/N_0	UV Dose (mJ/cm^2)		
	Batch 1	Batch 2	Mean Value from Literature (From Table 2.2)
1	30	19	24
2	51	32	37
3	71	45	46
4	92	58	49

The values of the multi-target model parameters determine for batch 2 spores were number of targets, $n_c = 3$ and rate constants, $k_m = 0.18$ cm^2/mJ. These parameter values differed from those determined for the batch 1 spores ($n_c = 3$, $k_m = 0.13$ cm^2/mJ, Table 4-2). Figure 4-2 shows the difference between inactivation of the batch 1 and batch 2 *B. subtilis* Spores preparations. The value of n_c and k_m for batch 2 spores were closer to those reported in the literature than were those for batch 1. So, the second batch preparation of *B. subtilis* spores was used in all subsequent experiments in this study.

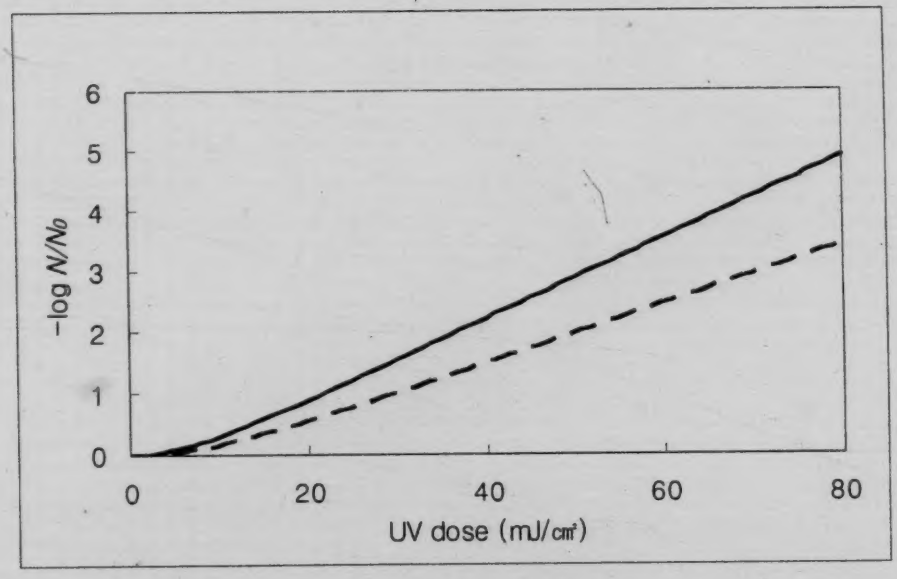

Figure 4-2. Comparison between UV inactivation of batch 1 and batch 2 *B. subtilis* spores suspended in de-ionized water. The multi-target model prediction of UV of Batch 1 and Batch 2 spores are shown by the broken line and solid line, respectively.

Therefore, we can conclude that different batches of spores yielded different UV inactivation results.

4.1.2 MS2 Coliphage

UV inactivation experiments were carried out with MS2 coliphage in DI water. The absorption coefficient of DI water after addition of MS2 coliphage ranged from 0.025 to 0.032 cm^{-1}.

Because some coliphages attached to their bacterial hosts via surface proteins, the proteins can be damaged by shear forces. UV inactivation experiments with MS2

coliphage included an elution and blending step instead of the more vigorous homogenization step.

Table 4-4 describes the UV irradiation conditions for MS2 coliphage in DI water. From the results of UV exposure trials of MS2 coliphage suspended in DI water are shown in Figure 4-3. The log inactivation of the MS2 prepared in this study was similar to that reported in the literature (see Table 2-3). Detailed results of the UV inactivation experiments are provided in Table A-3 in Appendix A. At 60 mJ/cm^2, the average log inactivation for experiments number 1 to 3 and experiments number 4 to 6 were 3.2 and 2.9, respectively. These values are very close to those (average 3.12 log inactivation) reported in the literature (Rauth, 1965; Havelaar et al., 1990; Wilson et al., 1992; Nieuwstad and Havelaar, 1994; Meng and Gerba, 1996; Tree et al., 1997; Sommer et al., 2001; Nunaualsuwan et al., 2002).

Multi-target model parameters determined by the least squares method for the inactivation of spores in DI water are provided in Table 4-5.

Table 4-4. Description of experimental conditions for UV exposure of MS2 coliphage suspended in DI water.

Experiment Number	Condition
1	Blended before UV exposure
2	Blended before UV exposure
3	Blended before UV exposure
4	Blended after UV exposure
5	Blended after UV exposure
6	Blended after UV exposure

A two-factor Analysis of Variance (ANOVA) test conducted on the two conditions indicated that there was no statistical difference in UV inactivation of MS2 blended before exposure versus after exposure at a 95% confidence level (p-value = 0.952, n = 30). The non-linear regression fit of the multi-target model (Equation 2.8) to inactivation data is shown in Figure 4-3. Multi-target model parameters determined by non-linear least squares regression for the inactivation of MS2 coliphage in DI water are provided in Table 4-5. The Microsoft® Excel® tool was used to minimize the sum of squares of the predicted and experimental values to obtain model parameters.

Table 4-5. Least-Squares Multi-target parameters, number of target, n_c and rate constant, k_m of multi-target model fitted to UV inactivation curves MS2 coliphage in DI water.

Experiment Number	Number of targets, n_c	Rate constants, k_m
1	1	0.1389
2	1	0.1206
3	1	0.1195
4	1	0.1482
5	1	0.1260
6	1	0.1112
Average	1	0.1274

Because MS2 coliphage is a single-stranded RNA phage, the one-hit one-target theory applies to MS2 coliphage. Under the one-hit one-target theory, one hit is assumed to inactivate a cell. As a result, the inactivation curve is linear and follows the Chick-Watson law, as shown in Figure 4-3. This result has been observed to be

the case for most single-stranded DNA and single-stranded RNA in viruses (Harm, 1980).

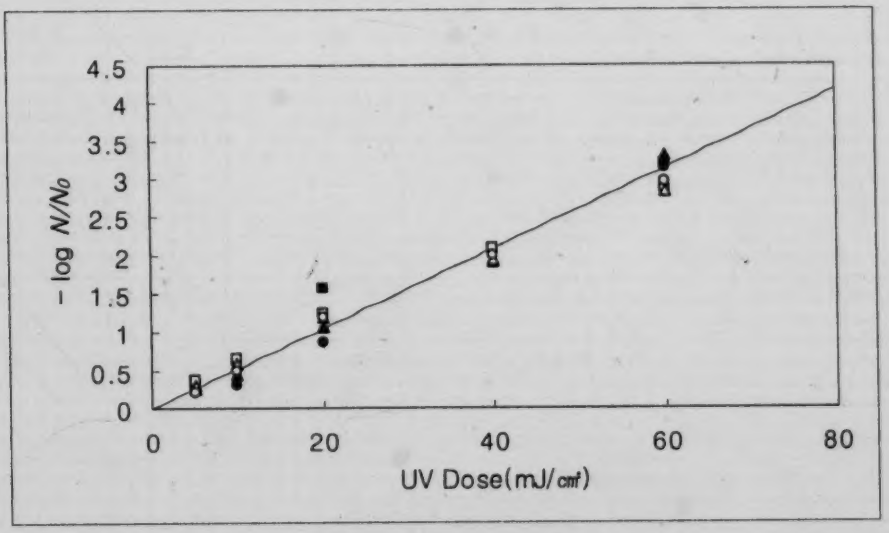

Figure 4-3. A multi-target Model predicted MS2 coliphage inactivation vs measured MS2 coliphage inactivation in DI water. Multi-target model (line), experiment Number 1 (■), experiment Number 2 (▲), experiment Number 3 (●), experiment Number 4 (□), experiment Number 5 (△), and experiment Number 6 (○) as described in Table 4-4.

4.2 IMPACT OF UPSTREAM TREATMENT PROCESSES ON UV INACTIVATION OF *B. SUBTILIS* SPORES

B. subtilis spores were seeded directly into each of the five samples of the raw river waters (See Table 3.2) and were then exposed to simulated coagulation,

flocculation, settling and filtration conditions using the jar test procedure followed by lab-scale anthracite-sand filtration. Three simulated treatment conditions were examined for each water-based test on the turbidity in the filtered water: an optimal treatment condition (0.3 NTU), a sub-optimal treatment condition (1 NTU) and poor treatment condition (2 NTU). The treatment conditions were achieved by varying the alum dose in the coagulation step of the jar test procedure.

It was expected that some of the spores in the filtered water would be aggregated with other spores or particle matter. In the pour plate technique, aggregates that might contain many spores would form a single colony and would be counted as a single spore resulting in undercounting of the spores. In order to measure the number of viable spores in the filtered water samples more accurately, the samples were homogenized either before or after exposure to UV doses of 0, 15, 40 mJ/cm^2 in order to break-up the aggregates and disperse the spores. In samples that were homogenized before UV exposure, the spores were more dispersed in the suspension, and all the spores were more likely to be exposed to the same UV dose. However, in samples that were homogenized after UV exposure, spores that may have been aggregated or engulfed in floc may not have received the same UV dose as dispersed spores. In this way, the effect of microbial aggregation in the filtered water on the UV inactivation might be compared. All UV exposures were done in triplicate.

Replicate experiments were carried out at two settings of UV dose based on principles of experimental design. The objective was to choose settings of the independent variables (UV Dose) that yielded the most accurate parameters of the multi-target UV inactivation model with the fewest number of measurements. The

criteria for doing this were to choose independent variable settings such that the size of the joint confidence region of the model parameters, n_c, and k_m, was minimized. This is called "Box and Lucas" criteria. More details of the determination of the optimal settings are provided in Appendix E. In Preliminary experiments, the two experimental UV doses settings were 12 and 50 mJ/cm^2. In NSR and BR water collected in winter, and RDR and NSR water collected in spring run-off, the two experimental dose settings were 15 and 40 mJ/cm^2. Replicate experiments at these doses were carried out in random order.

This section is further subdivided into preliminary experiments with NSR water, experiments conducted using NSR water and BR water collected during winter, and experiments conducted with NSR water and RDR water during spring run-off conditions. Table 4-6 describes the experimental conditions for each UV exposure experiment for *B. subtilis* spores. For each river water sample, the three levels of simulated water treatment (optimal coagulation, sub-optimal coagulation and poor coagulation) were tested. For each water treatment simulation condition, the samples of filtered water collected were either homogenized before UV exposure or after UV exposure. These six conditions were tested for each of the two UV does. Each UV exposure was carried out in triplicate. Therefore, for each river water sample, a total of 6 x 2 x 3 = 36 UV exposures were carried out. The concentration of live spores in the suspension after UV exposure, N, was determined for each exposure. In addition, a control sample (zero UV dose) was enumerated for each condition to determined N_0, the concentration of spores in suspension without UV exposure. Log inactivation was calculated as $-\log N/N_0$ for each UV exposure and an average log inactivation

was reported. The SYSTAT 11® ANOVA tool was used to calculate the ANOVA tests, using a 95% confidence level.

Table 4-6. Description of experiment conditions for UV inactivation of *B. subtilis* spores in Filtered water

Experimental Condition	Simulated Water Treatment Condition	Homogenization Step	As illustrated on Figures 4-4 to 4-8
1	Optimal coagulation	Before UV exposure	●
2	Sub-optimal coagulation	Before UV exposure	■
3	Poor coagulation	Before UV exposure	▲
4	Optimal coagulation	After UV exposure	○
5	Sub-optimal coagulation	After UV exposure	□
6	Poor coagulation	After UV exposure	△

4.2.1 Preliminary Experimental Results with NSR Water

A set of experiments was carried out to test the experimental protocol. The results of UV exposure trials carried out with a sample of NSR water collected during winter conditions are presented in Figure 4-4. Experiments were carried out at each of the conditions described in Table 4-6 for each of the two experimental UV doses (12 and 50 mJ/cm^2). The raw data are provided in Table B-1 in Appendix B. Multi-target model parameters determined by least squares regression for the inactivation of spores in filtered water are provided in Table 4-7.

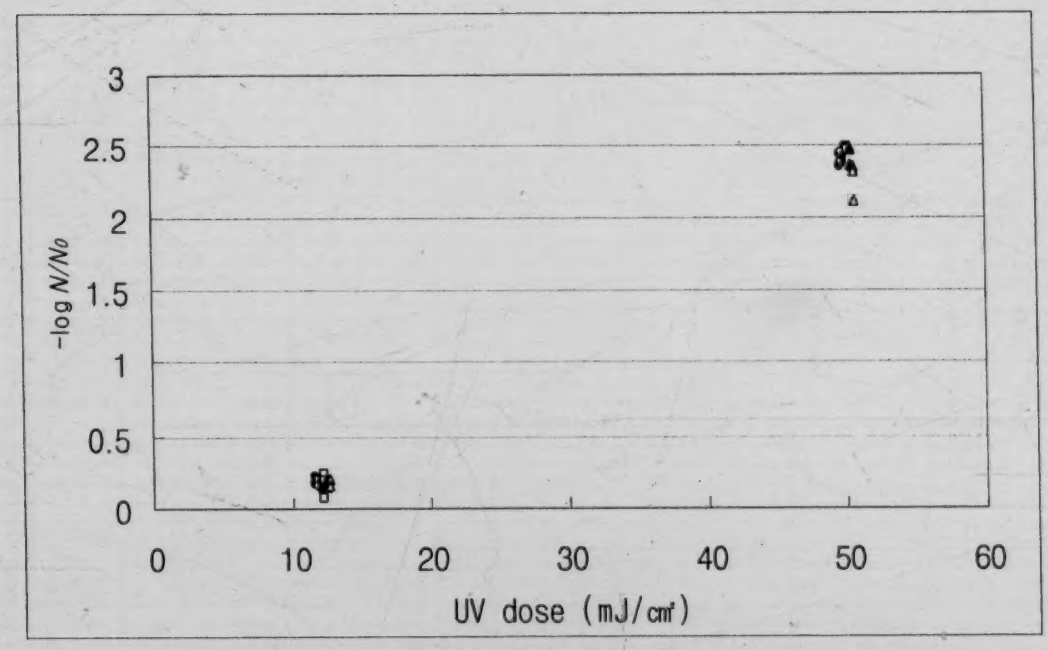

Figure 4-4. UV inactivation of *B. subtilis* spores seeded into NSR water during winter conditions at preliminary experiment and subjected to three simulated water treatment conditions for homogenization. Each datum represents the average inactivation of three exposures.

Legend:

- ● optimal coagulation, homogenized before UV exposure
- ■ sub-optimal coagulation, homogenized before UV exposure
- ▲ poor coagulation, homogenized before UV exposure
- ○ optimal coagulation, homogenized after UV exposure
- □ sub-optimal coagulation, homogenized after UV exposure
- △ poor coagulation, homogenized after UV exposure

Table 4-7. Least-Squares values of multi-target model parameters fitted to UV inactivation curves of *B. subtilis* spores in filtered NSR water.

Experimental Number (See Table 4-6)	Least-Square Multi-Target Model Parameters	
	Number of targets, n_c	Rate constants, k_m
1	6	0.1485
2	7	0.1512
3	6	0.1455
4	6	0.1434
5	7	0.1534
6	6	0.1430
DI water	3	0.1786

Each UV inactivation curve was characterized by a shoulder region at low UV dose followed by a region approximating first-order inactivation at higher UV dose. The effect of aggregation on the measured UV inactivation characteristics of the spores is not apparent in these experiments. In Figure 4-4, the inactivation of the spores in samples that were homogenized before UV exposure (H+UV) is almost the same as the inactivation of the spores in samples that were homogenized after UV exposure (UV+H). At 50 mJ/cm^2, the inactivation of spores in filtered water samples that were H+UV ranged from 2.3 to 2.5 log units, and the inactivation of spores in filtered water samples that were UV+H ranged from 2.1 to 2.5 log units.

Table 4-7 shows the parameters of the multi-target model, number of targets (n_c) and inactivation rate constant (k_m), used to fit the inactivation data. The value of n_c and k_m for all samples are similar. The value of n_c ranged from 6 to 7 and the value of k_m ranged from 0.14 to 0.16 cm^2/mJ. The inactivation rate constant, k_m, of clean

spores dispersed into DI water of 0.18 cm^2/mJ was similar to the inactivation rate constant of spores suspended in deionized that was H+UV and UV+H. The explanation for this observation is perhaps that in the deionized water matrix, the spores did not aggregate and were not embedded within any particles. A three-factor ANOVA test on the Figure 4-4, where the three factors were UV dose, filtration condition and homogenization procedure (i.e. before or after UV exposure) indicated that only UV dose was statistically significant (p-value = 1.06×10^{-11}, n=36). Filtration condition and the homogenization procedure were not statistically different, with filtration condition p-value = 0.134, n=36, and the homogenization procedure p-value = 0.245, n=36. The result suggests that aggregation of the spores was not affecting UV inactivation to a significant extent.

4.2.2 Experiments Carried out with River Water Collected during Mid Winter

The results of UV exposure trials carried out with NSR water and BR water collected during winter conditions are presented in Figure 4-5 and Figure 4-6, respectively. Experiments were carried out at each of the conditions described in Table 4-6 for each of the two experimental UV doses (15 and 40 mJ/cm^2). The raw data are provided in Table B-2 and Table B-3 in Appendix B, respectively. Multi-target model parameters determined by least squares for the inactivation of spores in NSR and BR water are provided in Table 4-8 and Table 4-9, respectively.

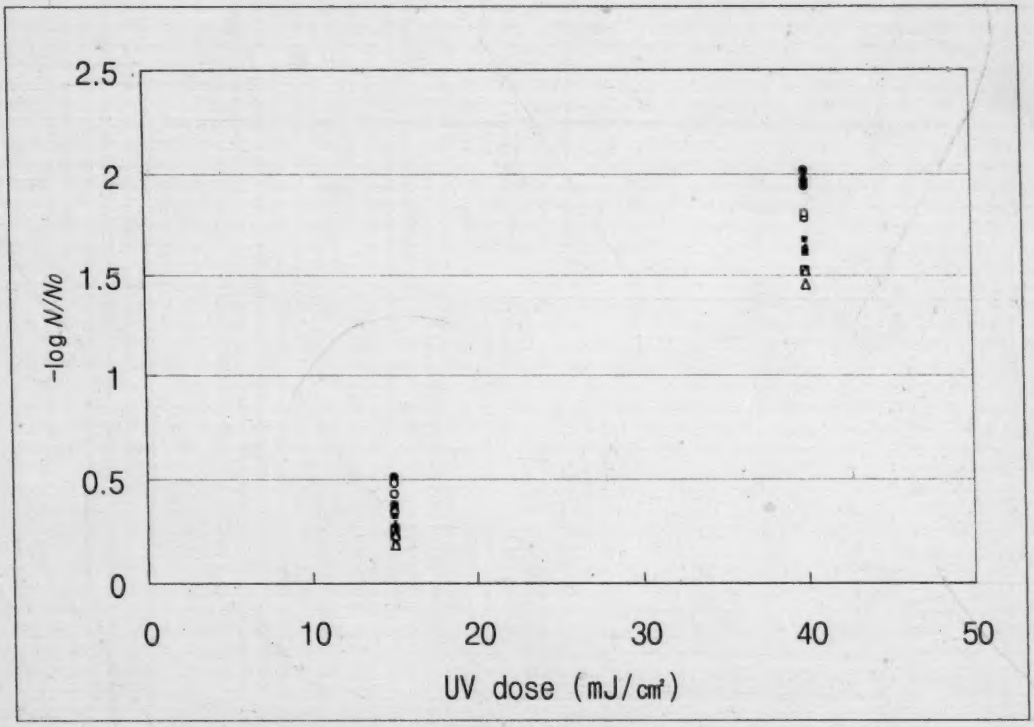

Figure 4-5. UV inactivation of *B. subtilis* spores seeded into NSR water during winter conditions and subjected to three simulated water treatment conditions for homogenization. Each datum represents the average inactivation of three exposures.

Legend:

- ● optimal coagulation, homogenized before UV exposure
- ■ sub-optimal coagulation, homogenized before UV exposure
- ▲ poor coagulation, homogenized before UV exposure
- ○ optimal coagulation, homogenized after UV exposure
- □ sub-optimal coagulation, homogenized after UV exposure
- △ poor coagulation, homogenized after UV exposure

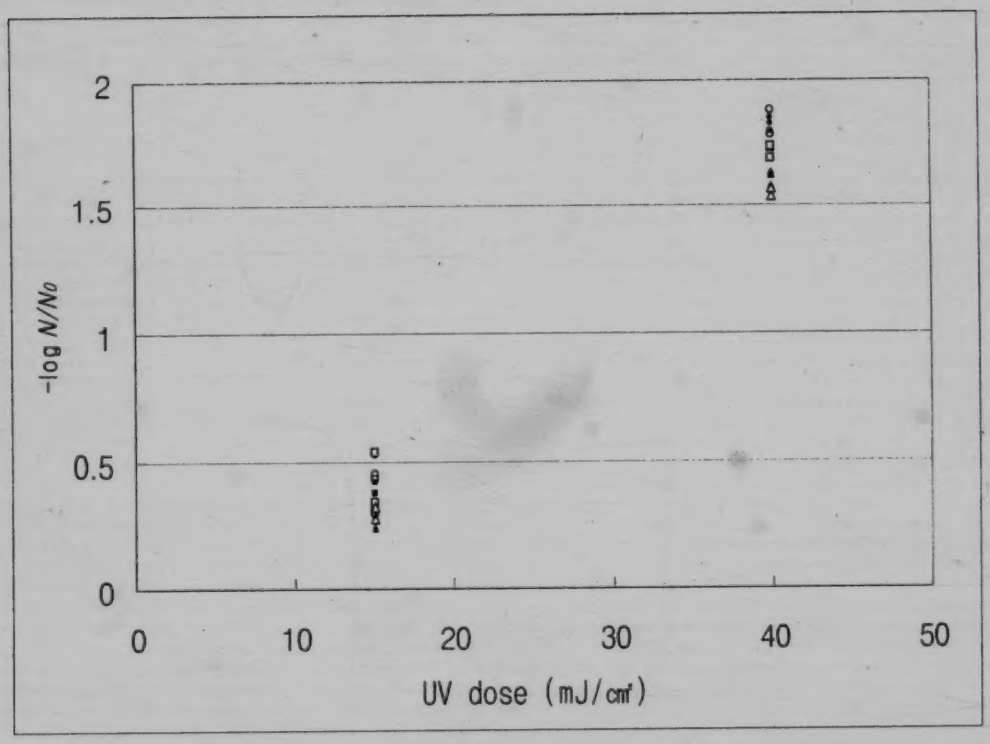

Figure 4-6. UV inactivation of *B. subtilis* spores seeded into BR water during winter conditions and subjected to three simulated water treatment conditions for homogenization. Each datum represents the average inactivation of three exposures.

Legend:

- ● optimal coagulation, homogenized before UV exposure
- ■ sub-optimal coagulation, homogenized before UV exposure
- ▲ poor coagulation, homogenized before UV exposure
- ○ optimal coagulation, homogenized after UV exposure
- □ sub-optimal coagulation, homogenized after UV exposure
- △ poor coagulation, homogenized after UV exposure

Table 4-8. Least-Squares values of multi-target model parameters fitted to UV inactivation curves of *B. subtilis* spores in filtered NSR water.

Experimental Number (See Table 4-6)	Least-Square Multi-Target Model Parameters	
	Number of targets, n_c	Rate constants, k_m
1	5	0.1562
2	5	0.1311
3	7	0.1437
4	3	0.1258
5	5	0.1244
6	6	0.1302
DI water	3	0.1786

Table 4-9. Least-Squares values of multi-target model parameters fitted to UV inactivation of *B. subtilis* spores in filtered BR water.

Experimental Number (See Table 4-6)	Least-Square Multi-Target Model Parameters	
	Number of targets, n_c	Rate constants, k_m
1	4	0.1433
2	4	0.1351
3	5	0.1328
4	3	0.1255
5	3	0.1215
6	4	0.1210
DI water	3	0.1786

Each UV inactivation curve was characterized by a shoulder region at low UV dose followed by a region approximating first-order inactivation at higher UV. The effect of aggregation on the measured UV inactivation characteristics of the spores is apparent by comparing spore inactivation for suspensions that were H+UV to spore inactivation for suspensions that were UV+H. Comparing the inactivation curves of

preliminary and winter conditions, there is more variation in winter conditions, but the curves are similar. In a preliminary experiment the samples that were H+UV and the samples that were UV+H were statistically the same. However, in winter conditions the UV inactivation of spores in samples that were H+UV was noticeably greater than that of spores in samples that were UV+H. At 40 mJ/cm^2, the inactivation of spores in winter condition water samples at optimal coagulation conditions and that were H+UV ranged from 1.8 to 2.0 log units whereas samples in poor condition that were UV+H ranged from 1.4 to 1.6 log units. Table 4-8 and Table 4-9 show the parameters, of the multi-target model, the number of targets (n_c) and inactivation rate constant (k_m), used to fit the inactivation data. The value of n_c ranged from 3 to 7 and the value of k_m ranged from 0.12 to 0.16 cm^2/mJ. The preliminary experiment's k_m value that was H+UV in optimal condition is similar to the preliminary experiment's k_m value that was UV+H in poor condition. The inactivation rate constant, k_m of clean spores dispersed into DI water 0.18 cm^2/mJ, which is also comparable to the inactivation rate constant of spores in winter condition's filtered water that was H+UV 0.15 cm^2/mJ.

Table 4-10 and Table 4-11 show the summary of a three-factor ANOVA test on Figure 4-5 and Figure 4-6 results, respectively.

Table 4-10. Results of three-factor ANOVA of UV experiments with *B. subtilis* spores in with NSR water collected in mid winter. (n=36)

Factor	Levels	p – value	Significance at 95% Confidence
UV Dose	15 or 40 mJ/cm^2	1.09×10^{-11}	S
Homogenization	Before UV / After UV	7.17×10^{-6}	S
Coagulation	Optimal / Sub-Optimal /Poor	1.46×10^{-11}	S

S – Significant at the 95% confidence level
NS – Not Significant at the 95% confidence level

Table 4-11. Results of three-factor ANOVA of UV experiments with *B. subtilis* spores in with BR water collected in mid winter. (n=36)

Factor	Levels	p – value	Significance at 95% Confidence
UV Dose	15 or 40 mJ/cm^2	1.19×10^{-11}	S
Homogenization	Before UV / After UV	3.39×10^{-2}	S
Coagulation	Optimal / Sub-Optimal /Poor	4.20×10^{-7}	S

S – Significant at the 95% confidence level
NS – Not Significant at the 95% confidence level

The explanation for this observation is maybe that some of spores present in the winter condition's filtered water were aggregated and embedded within particulate matter and were partially protected from UV radiation during the exposure.

In order to know the magnitudes of the differences in filtration condition at 40 mJ/cm^2, the paired t-test analyzed for NSR and BR water during mid-winter condition. Between optimal filter condition and sub-optimal filter condition the paired t-test analysis indicated that they were statistically different in both waters (NSR: p-value = 2.46×10^{-4}, n=12, BR: p-value = 3.28×10^{-5}, n=12), and between sub-optimal filter condition and poor filter condition the paired t-test analysis indicated

that there was no statistical difference between both waters (NSR: p-value = 0.724, n=12, BR: p-value = 0.062, n=12). It means that in just optimal filter conditions, the dose requirements for UV reactors will operate well to remove the microorganisms, but not in sub-optimal and poor filter conditions. Moreover, in sub-optimal and poor filter conditions, the level of aggregations and UV resistances are statistically same.

This result gives an indication of the rate of inactivation underestimation that may occur if aggregation and particle-association are not accounted for and different water matrix (turbidity) is correlated with the level of aggregation and UV resistance.

4.2.3 Experiments Carried out with River water Collected during Spring Run-off

The results of UV exposure trials carried out with RDR and NSR water collected during spring run-off conditions are presented in Figure 4-7 and Figure 4-8, respectively. Experiments were carried out at each of the conditions described in Table 4-6 for each of the two experimental UV doses (15 and 40 mJ/cm^2). The raw data are provided in Table B-4 and Table B-5 in Appendix B, respectively. Multi-target model parameters determined by least squares for the inactivation of spores in RDR and NSR water are provided in Table 4-12 and Table 4-13, respectively.

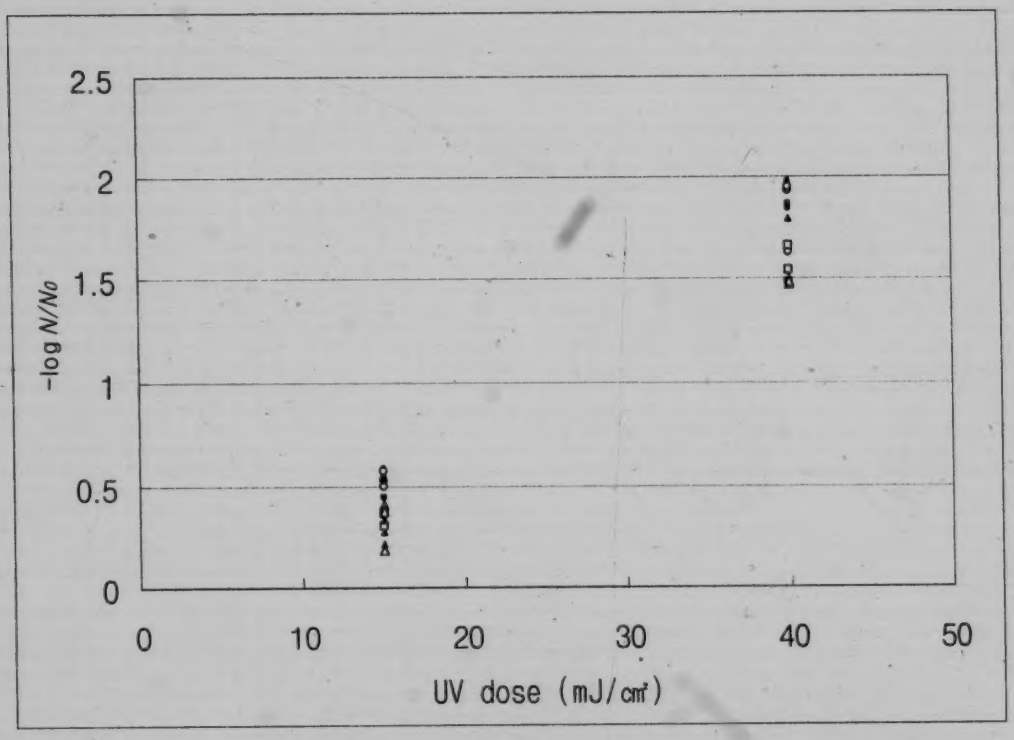

Figure 4-7. UV inactivation of *B. subtilis* spores seeded into RDR water during spring run-off conditions and subjected to three simulated water treatment conditions for homogenization. Each datum represents the average inactivation of three exposures.

Legend:

- ● optimal coagulation, homogenized before UV exposure
- ■ sub-optimal coagulation, homogenized before UV exposure
- ▲ poor coagulation, homogenized before UV exposure
- ○ optimal coagulation, homogenized after UV exposure
- □ sub-optimal coagulation, homogenized after UV exposure
- △ poor coagulation, homogenized after UV exposure

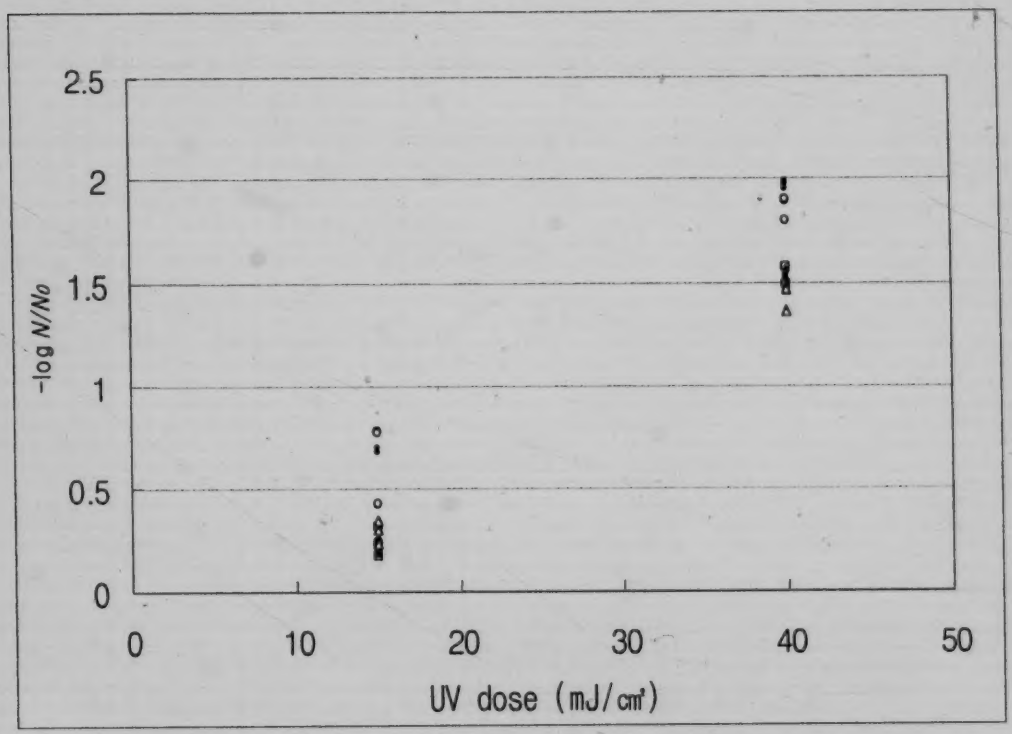

Figure 4-8. UV inactivation of *B. subtilis* spores seeded into NSR water during spring run-off conditions and subjected to three simulated water treatment conditions for homogenization. Each datum represents the average inactivation of three exposures.

Legend:

- ● optimal coagulation, homogenized before UV exposure
- ■ sub-optimal coagulation, homogenized before UV exposure
- ▲ poor coagulation, homogenized before UV exposure
- ○ optimal coagulation, homogenized after UV exposure
- □ sub-optimal coagulation, homogenized after UV exposure
- △ poor coagulation, homogenized after UV exposure

Table 4-12. Least-Squares values of multi-target model parameters fitted to UV inactivation of *B. subtilis* spores in filtered RDR water.

Experimental Number (See Table 4-6)	Least-Square Multi-Target Model Parameters	
	Number of targets, n_c	Rate constants, k_m
1	2	0.1286
2	3	0.1417
3	5	0.1303
4	2	0.1223
5	3	0.1144
6	3	0.1078
DI water	3	0.1786

Table 4-13. Least-Squares values of multi-target model parameters fitted to UV inactivation of *B. subtilis* spores in filtered NSR water.

Experimental Number (See Table 4-6)	Least-Square Multi-Target Model Parameters	
	Number of targets, n_c	Rate constants, k_m
1	2	0.1522
2	7	0.1316
3	5	0.1294
4	2	0.1375
5	6	0.1328
6	3	0.1052
DI water	3	0.1786

Each UV inactivation curve during spring run-off conditions was characterized by a shoulder region at low UV dose followed by a region approximating first-order inactivation at higher UV dose. The effect of aggregation on the measured UV inactivation characteristics of the spores is apparent by comparing spore inactivation for suspensions that were H+UV to spore inactivation for suspensions that were

UV+H. Compared with winter conditions and spring run-off conditions, the inactivation curves are almost the same. In the preliminary experiment the samples that were H+UV and the samples that were UV+H were statistically the same. However, in spring run-off conditions log reductions of the samples that were H+UV are significantly greater than log reductions of the samples that were UV+H. At 40 mJ/cm^2, the inactivation of spores in spring run-off condition water samples in optimal filtration condition that were H+UV ranged from 1.9 to 2.5 log units, whereas samples in poor filtration condition that were UV+H ranged from 1.3 to 1.6 log units. Table 4-12 and Table 4-13 show the parameters, number of targets (n_c) and inactivation rate constant (k_m) of the multi-target model used to fit the inactivation data. The value of n_c ranged from 2 to 7 and the value of k_m ranged from 0.10 to 0.18 cm^2/mJ. The inactivation rate constant, k_m of clean spores dispersed into DI water 0.18 cm^2/mJ, is also the same as the inactivation rate constant of spores in spring run-off condition water that was H+UV (0.18 cm^2/mJ).

Table 4-14 and Table 4-15 show the summary of a three-factor ANOVA test on Figure 4-7 and Figure 4-8 results, respectively.

Table 4-14. Results of three-factor ANOVA of UV experiments with *B. subtilis* spores in with RDR water collected in spring run-off. (n=36)

Factor	Levels	p – value	Significance at 95% Confidence
UV Dose	15 or 40 mJ/cm^2	1.33×10^{-11}	S
Homogenization	Before UV / After UV	8.09×10^{-4}	S
Coagulation	Optimal / Sub-Optimal /Poor	4.90×10^{-8}	S

S – Significant at the 95% confidence level
NS – Not Significant at the 95% confidence level

Table 4-15. Results of three-factor ANOVA of UV experiments with *B. subtilis* spores in with NSR water collected in spring run-off. (n=36)

Factor	Levels	p – value	Significance at 95% Confidence
UV Dose	15 or 40 mJ/cm^2	1.43×10^{-11}	S
Homogenization	Before UV / After UV	2.53×10^{-3}	S
Coagulation	Optimal / Sub-Optimal /Poor	1.43×10^{-11}	S

S – Significant at the 95% confidence level
NS – Not Significant at the 95% confidence level

The explanation for this observation is also maybe that some spores present in spring run-off optimal condition's filtered water were aggregated or embedded within particulate matter and, thus these spores were partially protected from UV radiation during the exposure. In order to know the magnitudes of the differences in filtration conditions at 40 mJ/cm^2, the paired t-test analyzed for RDR and NSR water during spring run-off condition. Between the optimal filter condition and the sub-optimal filter condition, the paired t-test analysis indicated that there were statistical differences between both waters (RDR: p-value = 0.005, n=12, NSR: p-value = 2.79×10^{-6}, n=12), but between the sub-optimal filter condition and the poor filter condition, the paired t-test analysis indicated that there was no statistical difference in between either water (RDR: p-value = 0.078, NSR: n=12, p-value = 0.125, n=12). This shows the same tendency as mid-winter conditions, although magnitudes are different. This means that it is difficult to know the minimum filter performance criteria or safety factors after optimal coagulation condition for UV inactivation of *B. subtilis*. Moreover, in this study at sub-optimal and poor filtration conditions the effect of aggregations on UV resistances was statistically the same.

This result also gives an indication of rate of inactivation underestimation that may occur if aggregation and particle-association are not accounted for and different water matrix (turbidity) is correlated with the level of aggregation and UV resistance.

4.3 IMPACT OF UPSTREAM TREATMENT PROCESSES ON UV INACTIVATION OF MS2 COLIPHAGE

MS2 coliphages were also seeded directly into each of five samples of the raw river water (See Table 3.2) and were then exposed to simulated coagulation, flocculation, settling, and filtration conditions using the jar test procedure followed by lab-scale anthracite-sand filtration. Three simulated treatment conditions were examined for each water-based test on the turbidity in the filtered water; an optimal treatment condition (0.3 NTU), a sub-optimal treatment condition (1NTU) and a poor treatment condition (2NTU). The treatment conditions were achieved by varying the alum dose in the coagulation step of the jar test procedure. It was expected that some of the MS2 coliphage in the filtered water would be aggregated with other MS2 coliphages or particulate matter. In the elution solution (beef extract) technique (See 3.5.4), aggregates that might contain many MS2 coliphages would form a single colony and would be counted as a single MS2 coliphage resulting in undercounting of the MS2 coliphages. In order to measure the number of viable MS2 coliphage in the filtered water samples more accurately, the samples were blended either before or after exposure to UV doses of 0, 15, 40 mJ/cm^2 in order to break-up the aggregates and disperse the MS2 coliphage. In samples that were blended before UV exposure, the MS2 coliphages were more dispersed in the suspension, and all the MS2

coliphages were more likely to be exposed to the same UV dose. However, in samples that were blended after UV exposure, MS2 coliphages that may have been aggregated or engulfed in floc may not have received the same UV dose as dispersed MS2 coliphages. In this way, the effect of microbial aggregation in the filtered water on the UV inactivation might be compared. All UV exposures were done in triplicate.

Replicate experiments were carried out at two settings of UV dose based on principles of experimental design. The objective was to choose settings of the independent variables (UV Dose) that yielded the most accurate parameters of the multi-target UV inactivation model with the fewest number of measurements. The criteria for doing this were to choose independent variable settings such that the size of the joint confidence region of the model parameters, n_c, and k_m, was minimized. This is called "Box and Lucas" criteria. More details of the determination of the optimal settings are provided in Appendix E. However, if the number of target, n_c is one, "Box and Lucas" criteria have infinite value. So, this experiment has the same UV dose as *B. subtils spores*. Therefore, two points were 15 and 40 mJ/cm^2. Replicate experiments at these doses were carried out in random order.

This section is further subdivided into preliminary experiment with NSR water, experiments conducted using NSR and BR water collected in winter conditions, and experiments conducted with RDR and NSR water collected in spring run-off conditions. Table 4-16 describes the experimental conditions for each UV exposure experiment for MS2 coliphage. For each river water sample, the three levels of simulated water treatment (optimal coagulation, sub-optimal coagulation and poor coagulation) were tested. For each water treatment simulation conditions, the samples

of filtered water collected were either blended before UV exposure or after UV exposure. These six conditions were carried out for each of the two UV does. Each UV exposure was carried out in triplicate. Therefore, for each river water sample, a total of 6 x 2 x 3 = 36 UV exposures were carried out. The concentration of live MS2 coliphages in the suspension after UV exposure, N, was determined for each exposure. In addition, a control sample (zero UV dose) was enumerated for each condition to determined N_0, the concentration of MS2 coliphages in suspension without UV exposure. Log inactivation was calculated as $-\log N/N_0$ for each UV exposure and an average log inactivation was reported. The SYSTAT 11® ANOVA tool was used to calculate the ANOVA tests, and to have a 95% confidence level.

Table 4-16. Description of experiment conditions for UV inactivation of MS2 coliphage in Filtered water

Experimental Condition	Simulated Water Treatment Condition	Blending Step	As illustrated on Figures 4-9 to 4-13
1	Optimal coagulation	Before UV exposure	●
2	Sub-optimal coagulation	Before UV exposure	■
3	Poor coagulation	Before UV exposure	▲
4	Optimal coagulation	After UV exposure	○
5	Sub-optimal coagulation	After UV exposure	□
6	Poor coagulation	After UV exposure	△

4.3.1 Preliminary Experimental Results with NSR Water

The results of UV exposure trials carried out with NSR water collected during winter conditions are presented in Figure 4-9. Experiments were carried out at each of the conditions described in Table 4-16 for each of the two experimental UV doses (15 and 40 mJ/cm^2). The raw data are provided in Table C-1 in Appendix C. One-hit

one-target model parameters determined by least squares for the inactivation of MS2 coliphage in filtered water are provided in Table 4-17.

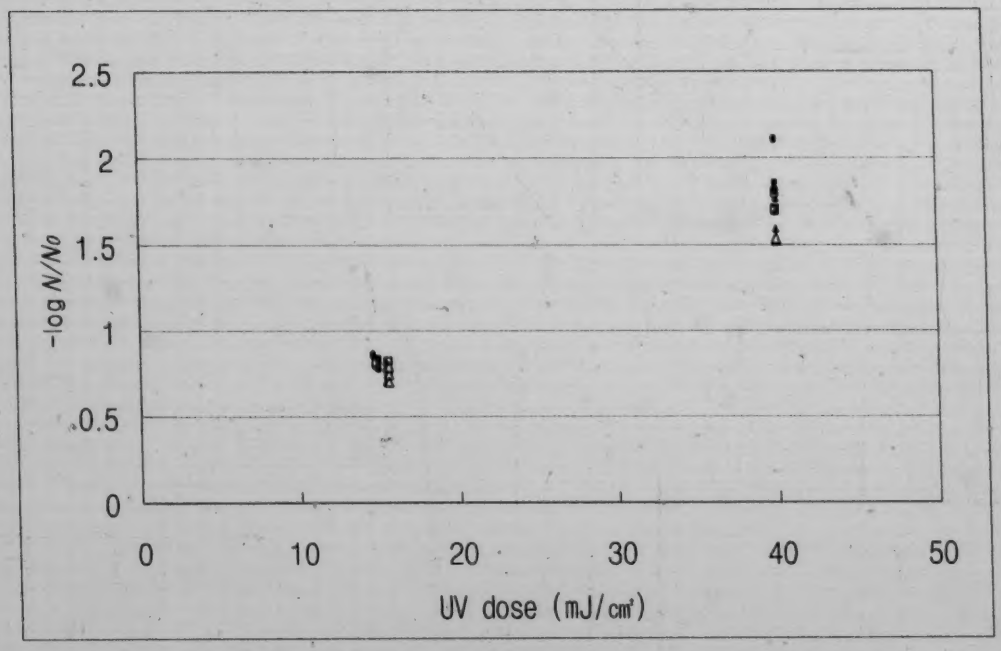

Figure 4-9. UV inactivation of MS2 coliphage seeded into NSR water during winter conditions at preliminary experiment and subjected to three simulated water treatment conditions for blending. Each datum represents the average inactivation of three exposures.

Legend:

- ● optimal coagulation, blended before UV exposure
- ■ sub-optimal coagulation, blended before UV exposure
- ▲ poor coagulation, blended before UV exposure
- ○ optimal coagulation, blended after UV exposure
- □ sub-optimal coagulation, blended after UV exposure
- △ poor coagulation, blended after UV exposure

Table 4-17. Least-Squares values of One-hit One-target model parameters fitted to UV inactivation curves of MS2 coliphage in filtered NSR water.

Experimental Number (See Table 4-12)	Least-Square One-hit One-target Model Parameters
	Rate constants, k_m
1	0.1285
2	0.1067
3	0.1024
4	0.1085
5	0.0995
6	0.0918
DI water	0.1274

All UV inactivation curves were characterized by linear regions as UV dose and the region of approximately first-order. This result has been observed to be the case for most single-stranded DNA and RNA in viruses like as MS2 coliphage. The effect of aggregation on the measured UV inactivation characteristics of the MS2 coliphage is apparent in these experiments by comparing MS2 coliphage inactivation that were blended before UV exposure (B+UV) to MS2 coliphage inactivation that were blended after UV exposure (UV+B). In Figure 4-9, for example, the inactivation of the MS2 coliphage in samples that were B+UV is noticeably greater than inactivation of the MS2 coliphage in samples that were UV+B. At 40 mJ/cm^2, the inactivation of MS2 coliphage in NSR filtered water samples that were B+UV at optimal condition ranged from 2.0 to 2.2 log units and the inactivation of MS2 coliphage in NSR filtered water samples that were UV+B at poor condition ranged from 1.4 to 1.6 log units. Table 4-17 shows the parameters of the one-hit one-target model, inactivation rate constant (k_m), used to fit the inactivation data. The value of k_m ranged from 0.09

to 0.13 cm^2/mJ. The inactivation rate constant, k_m of clean MS2 coliphage dispersed into DI water 0.13 cm^2/mJ was similar to the inactivation rate constant of MS2 coliphage in NSR filtered water at optimal condition that was B+UV (0.13 cm^2/mJ). However, comparing the inactivation rate constant of DI water and NSR filtered water at poor condition that was UV+B, the inactivation rate constant of NSR filtered water at poor condition that were UV+B is smaller than the inactivation rate constant of DI water (the inactivation rate constant of NSR filtered water at poor condition that were UV+B showed a value of 0.09 cm^2/mJ). The explanation for this observation is maybe that some of MS2 coliphage present in the NSR filtered water were aggregated and embedded within particulate matter and were partially protected from UV radiation during the exposure. Table 4-18 shows the summary of a three-factor ANOVA test on Figure 4-9 results.

Table 4-18. Results of three-factor ANOVA of UV experiments with MS2 coliphage in with NSR water in preliminary experiments. (n=36)

Factor	Levels	p – value	Significance at 95% Confidence
UV Dose	15 or 40 mJ/cm^2	1.14×10^{-11}	S
Blending	Before UV / After UV	1.33×10^{-9}	S
Coagulation	Optimal / Sub-Optimal /Poor	1.54×10^{-11}	S

S – Significant at the 95% confidence level
NS – Not Significant at the 95% confidence level

In order to know the magnitudes of the differences in filtration condition at 40 mJ/cm^2, the paired t-test analyzed for NSR water in preliminary experiments. Between optimal filter condition and sub-optimal filter condition the paired t-test

analysis indicated that there were totally statistically different (p-value = 8.00×10^{-5}, n=12), and between sub-optimal filter condition and poor filter condition the paired t-test analysis indicated that there were also a statistical difference (p-value = 3.96×10^{-5}, n=12). It means then, that in the *B. subtilis* case, the level of aggregations and UV resistances are statistically the same in sub-optimal and poor condition, however, in MS2 coliphage the level of aggregations and UV resistances were statistical differences. In *B. subtilis* it is difficult to know the minimum filter performance criteria or safety factors after optimal coagulation conditions; on the contrary, in MS2 coliphage it is easy to distinguish the minimum filter performance criteria or safety factors under different coagulation conditions.

This result gives an indication of the rate of inactivation underestimation that may occur if aggregation and particle-association are not accounted for. The level of coagulation is correlated with the effect of aggregation and UV resistance.

4.3.2 Experiments Carried out with River Water Collected during Mid Winter

The results of UV exposure trials carried out with NSR and BR water collected during winter conditions are presented in Figure 4-10 and Figure 4-11, respectively. Experiments were carried out at each of the conditions described in Table 4-16 for each of the two experimental UV doses (15 and 40 mJ/cm^2). The raw data are provided in Table C-2 and Table C-3 in Appendix C for NSR and BR waters, respectively. One-hit one-target model parameters determined by least squares for the UV inactivation of MS2 coliphage in NSR and BR water are provided in Table 4-19

and Table 4-20, respectively.

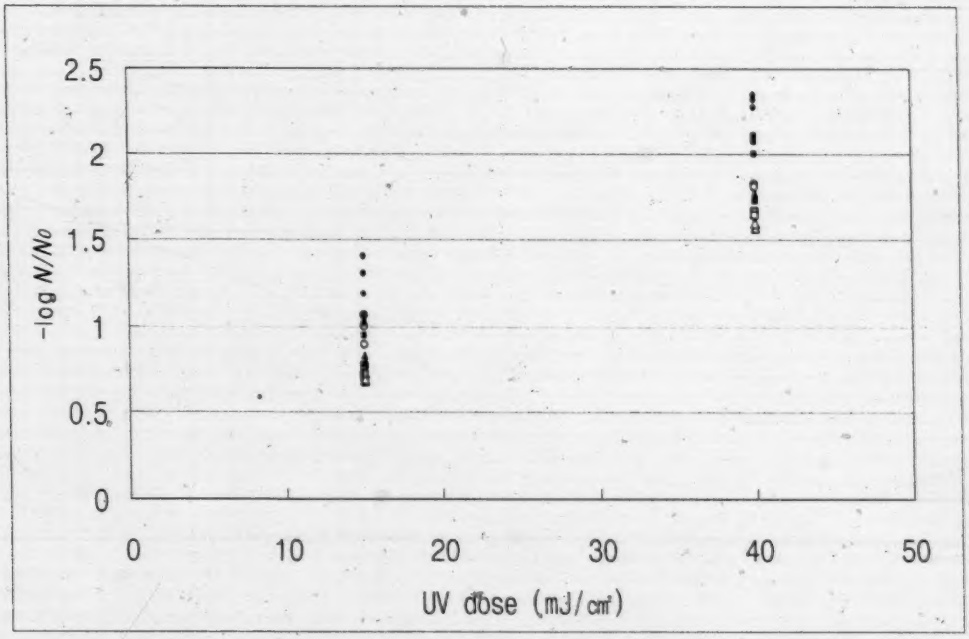

Figure 4-10. UV inactivation of MS2 coliphage seeded into NSR water during winter conditions and subjected to three simulated water treatment conditions for blending. Each datum represents the average inactivation of three exposures.

Legend:

- ● optimal coagulation, blended before UV exposure
- ■ sub-optimal coagulation, blended before UV exposure
- ▲ poor coagulation, blended before UV exposure
- ○ optimal coagulation, blended after UV exposure
- □ sub-optimal coagulation, blended after UV exposure
- △ poor coagulation, blended after UV exposure

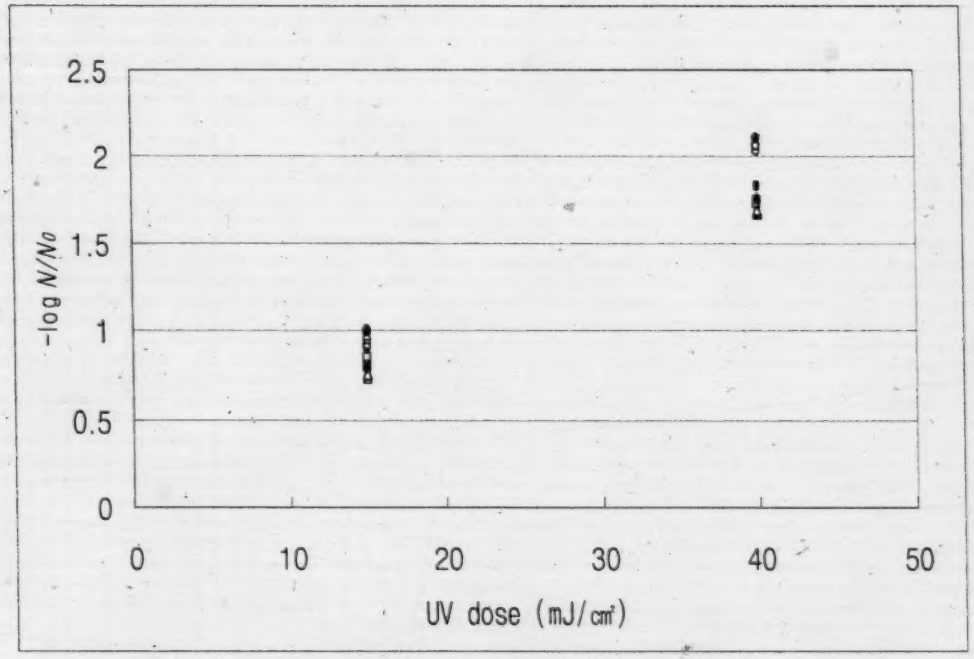

Figure 4-11. UV inactivation of MS2 coliphage seeded into BR water during winter conditions and subjected to three simulated water treatment conditions for blending. Each datum represents the average inactivation of three exposures.

Legend:

- ● optimal coagulation, blended before UV exposure
- ■ sub-optimal coagulation, blended before UV exposure
- ▲ poor coagulation, blended before UV exposure
- ○ optimal coagulation, blended after UV exposure
- □ sub-optimal coagulation, blended after UV exposure
- △ poor coagulation, blended after UV exposure

Table 4-19. Least-Squares values of One-hit One-target model parameters fitted to UV inactivation curves of MS2 coliphage in filtered NSR water.

Experimental Number (See Table 4-12)	Least-Square One-hit One-target Model Parameters
	Rate constants, k_m
1	0.1345
2	0.1182
3	0.1057
4	0.1094
5	0.1004
6	0.0937
DI water	0.1274

Table 4-20. Least-Squares values of One-hit One-target model parameters fitted to UV inactivation curves of MS2 coliphage in filtered BR water.

Experimental Number (See Table 4-12)	Least-Square One-hit One-target Model Parameters
	Rate constants, k_m
1	0.1234
2	0.1059
3	0.1002
4	0.1184
5	0.0994
6	0.0966
DI water	0.1274

All UV inactivation curves during winter conditions were characterized by a linear region as an UV dose and the region of approximately first-order. The effect of aggregation on the measured UV inactivation characteristics of the MS2 coliphage is apparent in winter conditions by comparing MS2 coliphage inactivation that were B+UV to MS2 coliphage inactivation that were UV+B. In winter conditions, the

inactivation of the MS2 coliphage in samples that were B+UV is noticeably greater than inactivation of the MS2 coliphage in samples that were UV+B. At 40 mJ/cm^2, the inactivation of MS2 coliphage in winter condition water samples that were B+UV at optimal condition ranged from 2.0 to 2.4 log units and the inactivation of MS2 coliphage in winter condition water samples that were UV+B at poor condition ranged from 1.4 to 1.6 log units. Table 4-19 and 4-20 show the parameters, the inactivation rate constant (k_m) of the one-hit one-target model used to fit the inactivation data. The value of k_m ranged from 0.09 to 0.14 cm^2/mJ. The inactivation rate constant, k_m of clean MS2 coliphage dispersed into DI water 0.14 cm^2/mJ, which is similar to the inactivation rate constant of MS2 coliphage in winter condition water at optimal condition that was B+UV (0.13 cm^2/mJ). However, comparing the inactivation rate constant of DI water and winter condition filtered water at poor condition that was UV+B, the inactivation rate constant of winter condition filtered water at poor condition that were UV+B is smaller than the inactivation rate constant of DI water (the inactivation rate constant of winter condition filtered water at poor condition that were UV+B showed a value of 0.09 cm^2/mJ). The explanation for this observation is maybe that some MS2 coliphage present in winter condition water were aggregated and embedded within particulate matter and were partially protected from UV radiation during the exposure.

Table 4-21 and Table 4-22 show the summary of a three-factor ANOVA test on Figure 4-10 and Figure 4-11 results, respectively.

Table 4-21. Results of three-factor ANOVA of UV experiments with MS2 coliphage in with NSR water collected in mid winter. (n=36)

Factor	Levels	p – value	Significance at 95% Confidence
UV Dose	15 or 40 mJ/cm^2	1.23×10^{-11}	S
Homogenization	Before UV / After UV	1.74×10^{-11}	S
Coagulation	Optimal / Sub-Optimal /Poor	1.35×10^{-11}	S

S – Significant at the 95% confidence level
NS – Not Significant at the 95% confidence level

Table 4-22. Results of three-factor ANOVA of UV experiments with MS2 coliphage in with BR water collected in mid winter. (n=36)

Factor	Levels	p – value	Significance at 95% Confidence
UV Dose	15 or 40 mJ/cm^2	1.07×10^{-11}	S
Homogenization	Before UV / After UV	8.42×10^{-6}	S
Coagulation	Optimal / Sub-Optimal /Poor	1.23×10^{-11}	S

S – Significant at the 95% confidence level
NS – Not Significant at the 95% confidence level

In order to know the magnitudes of the differences in filtration condition at 40 mJ/cm^2, the paired t-test analyzed for NSR and BR water during mid-winter condition. Between optimal filter condition and sub-optimal filter condition the paired t-test analysis indicated that they were statistically different in both waters (NSR: p-value = 0.029, n=12, BR: p-value = 9.99×10^{-16}, n=12), and between sub-optimal filter condition and poor filter condition the paired t-test analysis indicated that there were also statistically different in both waters (NSR: p-value = 0.003, n=12, BR: p-value = 2.38×10^{-4}, n=12). This shows the same tendency as preliminary experiment, although magnitudes are different.

This result also gives an indication of the rate of inactivation underestimation that may occur if aggregation and particle-association are not accounted for. The level of coagulation is correlated with the effect of aggregation and UV resistance.

4.3.3 Experiments Carried out with River water Collected during Spring Run-off

The results of UV exposure trials carried out with RDR and NSR water collected during spring run-off conditions are presented in Figure 4-12 and Figure 4-13, respectively. Experiments were carried out at each of the conditions described in Table 4-16 for each of the two experimental UV doses (15 and 40 mJ/cm^2). The raw data are provided in Table C-4 and Table C-5 in Appendix C, respectively. One-hit one-target model parameters determined by least squares for the UV inactivation of MS2 coliphage in RDR and NSR water are provided in Table 4-23 and Table 4-24.

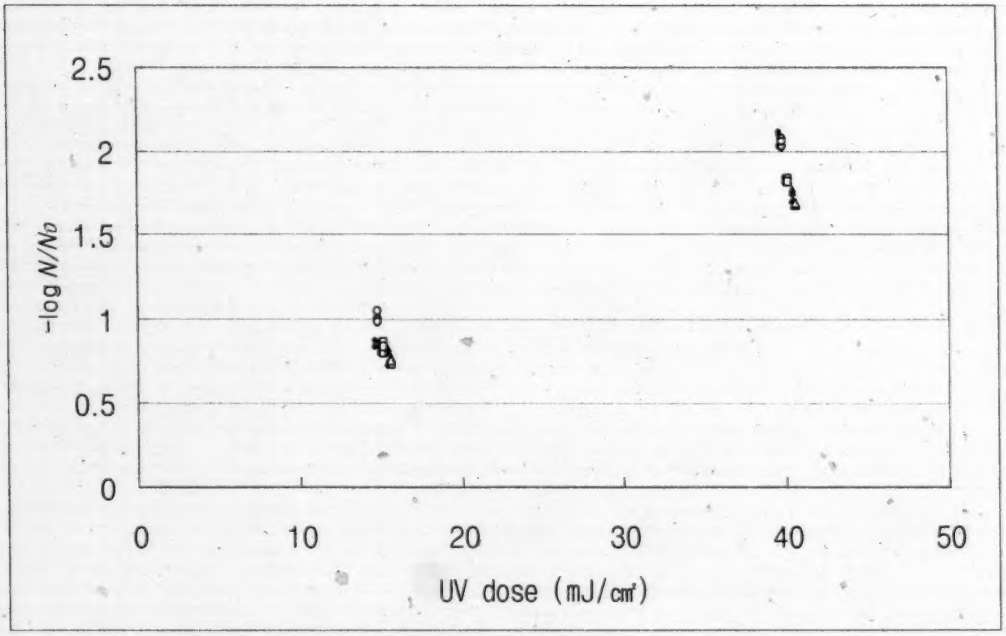

Figure 4-12. UV inactivation of MS2 coliphage seeded into RDR water during spring run-off conditions and subjected to three simulated water treatment conditions for blending. Each datum represents the average inactivation of three exposures.

Legend:

- ● optimal coagulation, blended before UV exposure
- ■ sub-optimal coagulation, blended before UV exposure
- ▲ poor coagulation, blended before UV exposure
- ○ optimal coagulation, blended after UV exposure
- □ sub-optimal coagulation, blended after UV exposure
- △ poor coagulation, blended after UV exposure

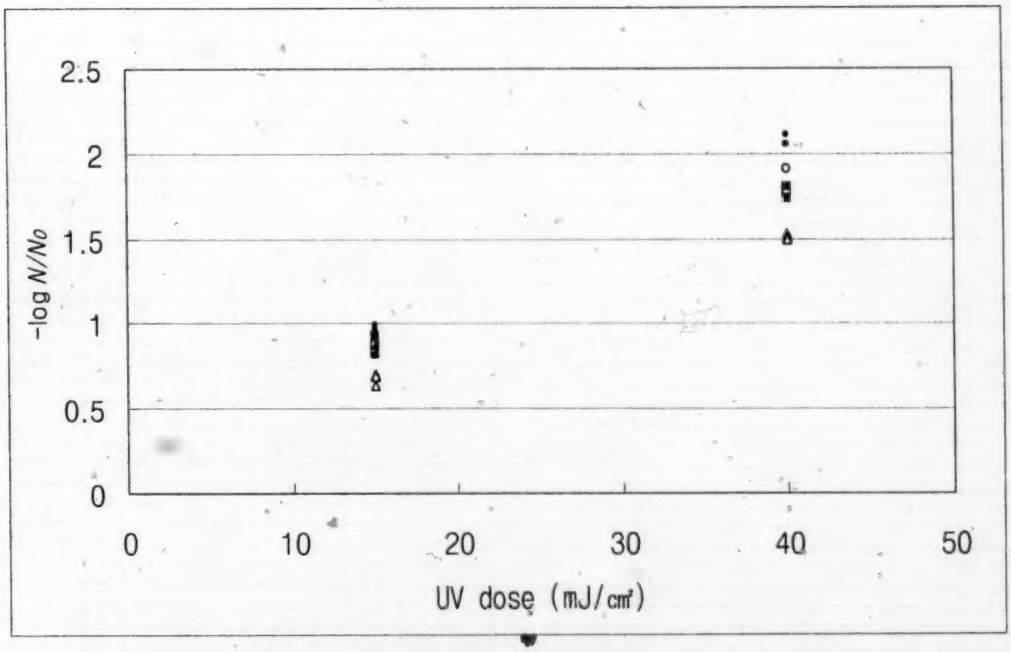

Figure 4-13. UV inactivation of MS2 coliphage seeded into NSR water during spring run-off conditions and subjected to three simulated water treatment conditions for blending. Each datum represents the average inactivation of three exposures.

Legend:

- ● optimal coagulation, blended before UV exposure
- ■ sub-optimal coagulation, blended before UV exposure
- ▲ poor coagulation, blended before UV exposure
- ○ optimal coagulation, blended after UV exposure
- □ sub-optimal coagulation, blended after UV exposure
- △ poor coagulation, blended after UV exposure

Table 4-23. Least-Squares values of One-hit One-target model parameters fitted to UV inactivation curves of MS2 coliphage in filtered RDR water.

Experimental Number (See Table 4-12)	Least-Square One-hit One-target Model Parameters
	Rate constants, k_m
1	0.1249
2	0.1064
3	0.1016
4	0.1175
5	0.1038
6	0.0968
DI water	0.1274

Table 4-24. Least-Squares values of One-hit One-target model parameters fitted to UV inactivation curves of MS2 coliphage in filtered NSR water.

Experimental Number (See Table 4-12)	Least-Square One-hit One-target Model Parameters
	Rate constants, k_m
1	0.1274
2	0.1207
3	0.1008
4	0.1053
5	0.0998
6	0.0872
DI water	0.1274

All UV inactivation curves during spring run-off conditions were characterized by a linear region as an UV dose and the region of approximately first-order. The effect of aggregation on the measured UV inactivation characteristics of the MS2 coliphage is apparent in spring run-off condition by comparing MS2 coliphage inactivation that were B+UV to MS2 coliphage inactivation that were UV+B. In

spring run-off conditions, the inactivation of the MS2 coliphage in samples that were B+UV is noticeably greater than inactivation of the MS2 coliphage in samples that were UV+B. At 40 mJ/cm^2, the inactivation of MS2 coliphage in spring run-off condition water samples that were B+UV at optimal condition ranged from 2.0 to 2.2 log units, and the inactivation of MS2 coliphage in Phase 4 filtered water samples that were UV+B at poor condition ranged from 1.4 to 1.7 log units. Table 4-23 and 4-24 show the parameters, inactivation rate constant (k_m) of one-hit one-target model used to fit the inactivation data. The value of k_m ranged from 0.08 to 0.13 cm^2/mJ. The inactivation rate constant, k_m of clean MS2 coliphage dispersed into DI water 0.12 cm^2/mJ, is similar to the inactivation rate constant of MS2 coliphage in spring run-off condition water at optimal condition that was B+UV (0.12 cm^2/mJ). However, comparing the inactivation rate constant of DI water and spring run-off condition filtered water at poor condition that was UV+B, the inactivation rate constant of spring run-off condition filtered water at poor condition that were UV+B is smaller than the inactivation rate constant of DI water (the inactivation rate constant of winter condition filtered water at poor condition that were UV+B showed a value of 0.08 cm^2/mJ). The explanation for this observation is maybe that some of MS2 coliphage present in spring run-off condition water were aggregated and embedded within particulate matter and were partially protected from UV radiation during the exposure. Table 4-25 and Table 4-26 show the summary of a three-factor ANOVA test on Figure 4-12 and Figure 4-13 results, respectively.

Table 4-25. Results of three-factor ANOVA of UV experiments with MS2 coliphage in with RDR water collected in spring run-off. (n=36)

Factor	Levels	p – value	Significance at 95% Confidence
UV Dose	15 or 40 mJ/cm^2	1.34×10^{-11}	S
Homogenization	Before UV / After UV	9.10×10^{-8}	S
Coagulation	Optimal / Sub-Optimal /Poor	5.12×10^{-9}	S

S – Significant at the 95% confidence level
NS – Not Significant at the 95% confidence level

Table 4-26. Results of three-factor ANOVA of UV experiments with MS2 coliphage in with NSR water collected in spring run-off. (n=36)

Factor	Levels	p – value	Significance at 95% Confidence
UV Dose	15 or 40 mJ/cm^2	1.12×10^{-11}	S
Homogenization	Before UV / After UV	1.77×10^{-11}	S
Coagulation	Optimal / Sub-Optimal /Poor	1.32×10^{-11}	S

S – Significant at the 95% confidence level
NS – Not Significant at the 95% confidence level

In order to know the magnitudes of the differences in filtration condition at 40 mJ/cm^2, the paired t-test analyzed for RDR and NSR water during spring run-off condition. Between the optimal filter condition and the sub-optimal filter condition the paired t-test analysis indicated that there were no statistical differences between both waters (RDR: p-value = 0.124, n=12, NSR: p-value = 0.198, n=12), but between sub-optimal filter condition and poor filter condition the paired t-test analysis indicated that there were statistical difference both waters (RDR: p-value = 1.34×10^{-5}, n=12, NSR: p-value = 7.71×10^{-7}, n=12). This signifier that in optimal and sub-optimal filter conditions dose requirements for UV reactors will operate well to

remove the microorganisms, not only in poor filter conditions. According to the paired t-test, in spring run-off river water the difference between optimal filtration condition and sub-optimal condition was not statistically significant, however, in winter river water the difference between optimal filtration condition and sub-optimal condition was statistically significant. One possible explanation is as follows: The turbidity of river water during spring run-off conditions is relatively high and a lot of alum and polymer is required for coagulation and flocculation (See Table 3-2). For river water during winter condition, the ratio of alum used in sub-optimal filter condition / amount of alum used in optimal filter condition of river water during spring run-off condition is 75%. For river water during winter condition, the ratio of the amount of alum used in sub-optimal filter condition / amount of alum used in optimal filter condition of river water during winter condition is 40%. This means that more MS2 coliphages were removed by coagulation, flocculation and filtration process during spring run-off conditions. However, in poor filter conditions the level of aggregations and UV resistances are statistically different.

This result also gives an indication of the rate of inactivation underestimation that may occur if aggregation and particle-association are not accounted for. The level of coagulation and resulting turbidity in the filtered water are correlated with the effect of aggregation and UV resistance.

5. DISCUSSION OF RESULTS

Ultraviolet (UV) disinfection has gained rapid popularity as a primary disinfectant of drinking water in North America. UV disinfection has been shown to inactivate most waterborne pathogens at relatively low, economical doses. However, this assumes that the UV light has a free path to the target organisms, which may not be the case when particles surround the organisms. In conventional drinking water processes, particles may be present in the filtered water for a number of reasons, including: (i) the source water experiences turbidity spikes, such as in shallow rivers in the spring, often at temporal intervals that are too short for the treatment processes to compensate, (ii) unstable coagulation, flocculation and clarification leads to carryover of floc to downstream treatment processes, or (iii) particle removal by filters is inadequate at certain times during the filter cycle (during the ripening phase and during breakthrough).

Previous research has shown that particles added directly to filtered and unfiltered water had no effect on the UV inactivation of microorganisms as long as the effect of reduced transmittance was factored into the dose calculation (Batch et al., 2004, and Passantino et al., 2004). Many waterborne outbreaks are known to have occurred during periods of sub-optimal operations of the filtration processes (Hrudey and Hrudey, 2004). In some cases, the failure of treatment systems to act as a barrier resulted in the outbreak to pathogenic microorganisms. Therefore, the objectives of this study were to further explore and better understand the phenomenon of microbial aggregation in filtered drinking water with the eventual goal of developing

recommendations to the water treatment industry.

The primary objective of this study was to evaluate the effect of upstream treatment processes on UV inactivation of *B. subtilis* and MS2 coliphage. In order to accomplish that objective, water treatment processes were simulated at bench-scale. Raw river water was spiked with *B. subtilis* and MS2 coliphage and was passed though that simulated process. *B. subtilis* and MS2 coliphage collected in the filtered water were exposed to UV irradiation using a collimated beam apparatus. The hypothesis was that *B. subtilis* and MS2 coliphage in filtered water might be in an aggregated state and that this can adversely affect the efficiency of UV disinfection. Therefore, it was extremely important to make sure that the state of aggregation of *B. subtilis* and MS2 coliphage in filtered water was not disturbed by any experimental artifact. Coagulation, flocculation, membrane filtration or any other means of reducing the volume of the filtered water was avoided because these techniques could potentially alter the state of aggregation of *B. subtilis* and MS2 coliphage. The collimated beam apparatus was preferred for the exposure experiments for two reasons; (i) the UV dose delivery in a collimated beam apparatus is well-defined and accurate, and (ii) the collimated beam apparatus allows for better physical control of the experimental procedures and containment of samples. Optimal, sub-optimal and poor coagulation conditions were intentionally simulated in this study in order to extend the coagulation conditions that were investigated in previous research that was based on only a single sub-optimal coagulation condition (Craik and Uvbiama, 2005).

The interpretation of results in this study in terms of UV disinfection performance

will provide useful information about the effects of aggregation on UV inactivation of two indicator microorganisms, *B. subtilis* and MS2 coliphage, in filtered water; the tests were done on indicator microorganisms only, and not on the actual pathogens. Table 5-1 shows the summary of UV inactivation of *B. subtilis*.

Table 5-1. Summary of UV inactivation of *B. subtilis*.

Season condition	Raw Water	UV dose (mJ/cm^2)	Homogenization before UV exposure			Homogenization after UV exposure		
			Optimal	Sub-opt	Poor	Optimal	Sub-opt	Poor
Winter	NSR	15	0.402	0.275	0.237	0.409	0.245	0.220
		40	1.971	1.640	1.632	1.789	1.518	1.495
	BR	15	0.408	0.364	0.283	0.407	0.385	0.293
		40	1.852	1.750	1.608	1.797	1.717	1.545
Spring Run-off	RDR	15	0.569	0.499	0.271	0.532	0.348	0.306
		40	1.977	1.857	1.580	1.831	1.557	1.483
	NSR	15	0.715	0.205	0.217	0.633	0.230	0.287
		40	1.969	1.516	1.527	1.864	1.518	1.415

In preliminary experiments with NSR water collected under winter condition for *B. subtilis* UV inactivation experiment, there was no statistical difference between UV inactivation of *B. subtilis* spores in filtered water that was homogenized before UV exposure (H+UV) and samples that were in filtered water that were homogenized after UV exposure (UV+H). The explanation for this observation is a low turbidity raw water such as preliminary experiments with NSR water collected under winter condition (2.2 NTU, see in Table 3-1), there was less opportunity for spores to aggregate and became embedded within particles during any filtration condition. However, in experiments with NSR and BR water collected under winter conditions and experiments with NSR and RDR water collected under spring run-off conditions,

the difference in log inactivation of *B. subtilis* between samples that were H+UV and samples that were UV+H was statistically significant. As mentioned earlier, some of the spores in filtered water that was UV+H were presumed to be in an aggregated state during UV exposure, whereas spores in filtered water that were H+UV were assumed to be in mono-dispersed state during UV exposure. From Table 5-1 at a dose of 40 mJ/cm^2, the average difference in log inactivation between samples that were in filtered water that were H+UV and samples that were in filtered water that were UV+H was 0.22, and this difference was statistically significant at the 95% confidence level (p-value = 0.008, n=72). This suggests that there was a significant reduction in UV inactivation of the spores that broke through the filter media compared to the spores that were mono-dispersed. However, although the 0.22 log difference was statistically different, it is not a large practical difference at the 2 to 3 log level of inactivation. From Table 5-1 at a dose of 40 mJ/cm^2, in every water tested the difference in log inactivation between optimal filtration condition samples and sub-optimal filtration condition samples was statistically significant with the average difference in log inactivation between optimal filtration condition samples and sub-optimal filtration condition samples being 0.29. This difference was statistically significant at the 95% confidence level when the results of the all waters were pooled (p-value = 1.45×10^{-11}, n=48).

In individual waters the difference in log inactivation of *B. subtilis* spores in filtered water between sub-optimal filtration conditions and poor filtration conditions samples was not statistically significant. However, the average log inactivation of *B. subtilis* spores at sub-optimal filtration conditions samples was 0.12 greater than poor

filtration conditions samples and this difference was statistically significant at the 95% confidence level when the results of the all waters were pooled (p-value = 0.003, n=48). It means that although there is not a statistical difference between sub-optimal condition and poor condition in individual waters, there is statistical difference when the results of the all waters were pooled. However, the difference of 0.29 and 0.12, even thought it was statistically significant, is not a practically significant difference.

There was no significant difference in UV inactivation of *B. subtilis* after optimal filtration condition between waters collected in winter condition and during spring run-off (p-value = 0.633, n=72).

For *B. subtilis* experiments, the log inactivation was less than best possible performance at optimal filtration conditions by factors of 1.07, 1.23 and 1.31 for plant performance at optimal filtration, sub-optimal filtration and poor filtration conditions, respectively; Best performance means H+UV because homogenization broke apart aggregates, so the spores in filtered water samples were more likely to be mono-dispersed, and more likely to be susceptible to UV inactivation; Real plant performance means UV+H, however in real plant there is no homogenization step, this step is strictly an experimental procedure. This suggests that it is necessary to take the aggregation into account during design of UV disinfection systems. This is less important during optimal filtration but is more important during poor filtration. In practice, however, it may be important to consider poor filtration episodes. All in all, the filtration condition might be a determinant of the extent of aggregation and the effect on UV inactivation systems.

Table 5-2 shows the summary of UV inactivation of MS2 coliphage.

Table 5-2. Summary of UV inactivation of MS2 coliphage.

Season condition	Raw Water	UV dose (mJ/cm^2)	Blended before UV exposure			Blended after UV exposure		
			Optimal	Sub-opt	Poor	Optimal	Sub-opt	Poor
Winter	NSR	15	1.295	1.006	0.795	0.978	0.727	0.697
		40	2.311	2.059	1.757	1.818	1.657	1.595
	BR	15	0.979	0.854	0.785	0.924	0.821	0.753
		40	2.082	1.830	1.746	2.053	1.733	1.683
Spring Run-off	RDR	15	0.951	0.882	0.856	0.994	0.755	0.717
		40	2.136	2.044	1.815	1.896	1.778	1.454
	NSR	15	0.985	0.909	0.844	0.928	0.844	0.676
		40	2.147	2.044	1.777	1.823	1.780	1.510

In preliminary experiments with NSR water collected under winter condition for MS2 coliphage UV inactivation experiment, there was a statistical difference between samples that were in filtered water that were blended before UV exposure (B+UV) and samples that were in filtered water that were blended after UV exposure (UV+B). This result is different from preliminary experiments with NSR water collected under winter condition for *B. subtilis*. MS2 coliphage are much smaller than *B. subtilis* spores (*B. subtilis* spores: 1.2 um to 1.5 um, MS2 coliphage: 0.01 um to 0.025um), so MS2 coliphage is more likely to be aggregated or clumped together. In experiments with NSR and BR water collected under winter conditions and experiments with NSR and RDR water collected under spring run-off conditions for MS2 coliphage UV inactivation experiment, the difference in log inactivation of MS2 coliphage between samples that were B+UV and samples that were UV+B was statistically significant. As mentioned earlier, some of MS2 coliphages in filtered water that were B+UV were presumed to be in an aggregated state during the UV exposure, whereas coliphage in filtered water that were B+UV were assumed to be in

mono-dispersed state. From Table 5-2 at a dose of 40 mJ/cm^2, the average difference in log inactivation between samples that were in filtered water that were B+UV and samples that were in filtered water that were UV+B was 0.24 and this difference was statistically significant at the 95% confidence level (p-value = 1.77×10^{-7}, n=72). This suggests that there was a significant reduction in UV inactivation of MS2 coliphage that broke through the filter media compared to MS2 coliphage that were mono-dispersed. However, although the 0.24 log difference was statistically different, it is not a large practical difference at the 2 to 3 log level of inactivation. From Table 5-2 at a dose of 40 mJ/cm^2, in every water tested the difference in log inactivation between optimal filtration condition samples and sub-optimal filtration condition samples was not statistically significant. However, the average difference in log inactivation between optimal filtration condition samples and sub-optimal filtration condition samples was 0.17 and this difference was statistically significant at the 95% confidence level when all waters were considered (p-value = 0.001, n=48). The average difference in log inactivation between sub-optimal filtration condition samples and poor filtration condition samples was 0.20, and this difference was also statistically significant at the 95% confidence level when all waters were considered (p-value = 1.79×10^{-5}, n=48). However, the difference of 0.17 and 0.20, even thought it was statistically significant, is not a practically significant difference.

For MS2 coliphage experiments, the log inactivation was less than best possible performance by factors of 1.14, 1.25 and 1.40 when optimal filtration conditions were compared to real plant performance at optimal filtration, sub-optimal filtration and poor filtration conditions, respectively; Best possible performance is assumed to

be when samples were B+UV, so that the MS2 coliphages in the filtered water was mono-dispersed and more susceptible to UV inactivation; Real plant performance is represented by those experiments where were UV+B, however, in real plant there is no blending step, this step is strictly an experimental procedure. This is less important during optimal filtration but is more important during poor filtration. In practice, however, it may be important to consider poor filtration episodes. All in all, the filtration condition might be a determination of the extent of aggregation and the effect on UV inactivation systems.

However, an ANOVA test conducted on all waters, where the factor was individual water, indicated that there was no statistical difference. From a statistical perspective there was no difference among preliminary experiment, experiments with NSR and BR water collected under winter conditions and experiments with NSR and RD River water collected under spring run-off conditions for *B. subtilis* UV inactivation (p-value = 0.960, n=144), and also no statistical difference among preliminary experiment, experiments with NSR and BR water collected under winter conditions and experiments with NSR and RDR water collected under spring run-off conditions for MS2 coliphage UV inactivation (p-value = 0.988, n=144). This suggests that even though mid-winter river water turbidity and spring run-off river water turbidity is different, usually the water beneath the ice is relatively low in turbidity and colour and during the spring run-off the turbidity and colour increase considerably (See Table 3-1), there was no significant difference between mid-winter river water and spring run-off river water, and no significant difference among raw

water sources. It means that the aggregation phenomenon is independent of the type of water and the season during. The effect of aggregation is mainly determined by the coagulation conditions and the type of microorganisms.

The findings of this study conflict with the findings of some earlier studies which reported that the UV inactivation of MS2 coliphage was independent of turbidity provided that absorbance of the water was taken into account in the determination of UV dose (Batch et al., 2004; Passantino et al., 2004). One possible explanation for the reported insignificant effects is that the particles and microorganisms used in those studies were added directly to filtered water. Hence, the microorganisms had little opportunity to be aggregated. No significant difference was observed between the inactivation of microorganisms dispersed in DI water and the inactivation of microorganisms added to filtered water. In the present study, *B. subtilis* and MS2 coliphage were spiked into raw water and the raw water was coagulated, flocculated, settled and filtered prior to UV exposure. These processes encouraged the aggregation of *B. subtilis* and MS2 coliphage in filtered water which in turns affected the efficiency of UV inactivation.

The findings of this study agree with the study conducted by Uvbiama and Craik (2005) and Mahmud (2006) where the investigators observed statistical significant reduction in UV inaction of *B. subtilis* and *C. parvum* oocysts in filtered water samples that were homogenized after UV exposure, compared to the samples that were homogenized before UV exposure. However, the magnitudes of log inactivation

are different; for example, Uvbiama and Craik (2005) said the log inactivation was underestimated by factors by 3.6, when the real plant performance was compared to the best performance. In this experiment the log inactivation was underestimated only by factors 1.3 in *B. subtilis*.

6. CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

The objective of this research was to further explore and better understand the phenomenon of microbial aggregation in filtered drinking water with the eventual goal of developing recommendations to the water treatment industry. The conclusions that could be reached from the findings of the study are as follows:

- Some of *B. subtilis* and MS2 coliphages that broke through the filter media during periods of sub-optimal and poor conditions were in an aggregated form, either as microbe-microbe or microbe-particle aggregates. This aggregation may have had a measurable and statistically significant effect on the apparent UV inactivation of microorganisms in the filtered water.

- Aggregation phenomenon is independent of the type of water and the season during.

- The effect of aggregation is mainly determined by the coagulation conditions and the type of microorganisms. Viruses are more likely to be aggregated or clumped together than bacteria or protozoans.

- The UV inactivation of *B. subtilis* spores was reduced by 0.22 log-unit at 40 mJ/cm^2 as a result of the aggregation. This reduction in UV inactivation at 40 mJ/cm^2 was statistically significant at the 95% confidence level (p-value = 0.008, n=72). The UV inactivation of MS2 coliphage was also reduced by

0.24 log-unit at 40 mJ/ cm^2 as a result of the aggregation. This reduction in UV inactivation at 40 mJ/cm^2 was as statistically significant at the 95% confidence level (p-value = 1.36×10^{-7}, n=72). These results suggest that the UV inactivation of *B. subtilis* and MS2 coliphage in poorly filtered water might be overestimated if the effect of *B. subtilis* and MS2 coliphage aggregation is not taken into consideration.

6.2 RECOMMENDATIONS

Some recommendations as a result of this study are

- The experimentally filtered water samples were exposed to UV using a batch UV exposure apparatus. The limitation of this approach is that the filtered water samples must be stored for a time period and manipulated prior to UV exposure and this may affect the state of aggregation. It is also a slow procedure, requiring much storage and sample manipulation. In order to offset the requirements a new and more efficient approach based on the use of a small-scale continuous-flow or pilot-scale continuous-flow UV reactor might be recommended. Both of these methods will address the efficiency requirements in terms of speed of testing and processing. The demands placed on the system to process contaminated raw river water in larger volumes may necessitate a more efficient method of testing and processing. The recommendation, therefore, is for a mobile, possibly truck-mounted, pilot-scale continuous-flow UV reactor consisting of a

coagulation and flocculation tank, a sedimentation tank, a flow meter, and a small point-of-use UV reactor equipped with a low-pressure mercury arc lamp.

- Because the aggregation is currently not considered in the UV dose (I x t) table provided in the US EPA UV guidance manual (USEPA, 1999), it is recommended that a safety factor of 1.4 be applied to the rate constant used for the design of future UV reactors. Although increasing the UV dose may not inactivate some microorganisms that are engulfed, there is need to recognize that the current values in the dose table need to account for aggregation.

- Other types of coagulants and polymers could be studied to determine if a change in the coagulant or polymers type would have any impact on the results of this study. This study used an alum coagulant; however, alum floc may have different characteristics from iron floc and this may impact experimental results.

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APPENDIX A EXPERIMENTAL DATA AND INFORMATION FROM CONTROL EXPERIMENTS

Table A-1. Data for UV inactivation of *B. subtilis* spores in DI water

Experiment Number[a]	Dose	Dilution	Count			Average count	CFU/mL	Log Inactivation
			1	2	3			
1	0	10^{-5}	71	60	65	65.5	6.55 E+06	
2		10^{-5}	63	64	65	64.0	6.40 E+06	
3		10^{-5}	60	65	63	62.5	6.25 E+06	
4		10^{-5}	42	46	44	44.0	4.40 E+06	
5		10^{-5}	42	41	42	41.5	4.15 E+06	
6		10^{-5}	239	245	263	251.0	2.51 E+07	
1	5	10^{-5}	56	48	52	52.0	5.20 E+06	0.100
2		10^{-5}	45	39	42	45.0	4.50 E+06	0.153
3		10^{-5}	39	38	37	45.0	4.50 E+06	0.143
4		10^{-5}	32	30	31	30.9	3.09 E+06	0.154
5		10^{-5}	27	27	28	26.9	2.69 E+06	0.188
6		10^{-5}	241	228	231	235.0	2.35 E+07	0.030
1	10	10^{-5}	36	59	43	47.5	4.75 E+06	0.104
2		10^{-5}	49	48	42	40.9	4.09 E+06	0.195
3		10^{-5}	40	35	37	37.5	3.75 E+06	0.222
4		10^{-5}	30	26	28	28.0	2.80 E+06	0.196
5		10^{-5}	25	26	25	25.7	2.57 E+06	0.208
6		10^{-5}	225	214	218	220.0	2.20 E+07	0.058
1	20	10^{-4}	220	200	211	210.0	2.10 E+06	0.494
2		10^{-4}	212	241	228	227.0	2.27 E+06	0.451
3		10^{-4}	175	204	184	190.0	1.90 E+06	0.518
4		10^{-4}	158	153	155	156.0	1.56 E+06	0.452
5		10^{-4}	163	138	145	151.0	1.51 E+06	0.441
6		10^{-5}	97	93	95	95.0	5.10 E+05	0.422
1	40	10^{-3}	90	120	106	105.0	1.05 E+05	1.700
2		10^{-3}	266	123	156	195.0	1.95 E+05	1.517
3		10^{-3}	189	209	194	200.0	2.00 E+05	1.497
4		10^{-3}	103	112	108	108.0	1.08 E+05	1.612
5		10^{-3}	127	116	105	116.0	1.16 E+05	1.554
6		10^{-4}	55	52	53	53.5	2.90 E+05	1.671
1	60	10^{-2}	33	51	74	53.5	5.35 E+03	3.088
2		10^{-2}	104	196	154	150.0	1.50 E+04	2.630
3		10^{-2}	276	268	270	270.0	2.70 E+04	2.361
4		10^{-2}	124	128	132	128.0	1.28 E+04	2.536
5		10^{-2}	183	152	168	167.0	1.67 E+04	2.395
6		10^{-3}	72	71	71	71.5	7.15 E+04	2.545

[a] See Table 4-1.

Table A-2. Data for UV inactivation of the second batch preparation's *B. subtilis* spores in DI water

Dose	Dilution	Count			Average count	CFU/mL	Log Inactivation
		1	2	3			
0	10^{-5}	70	76	72	73.0	7.30 E+06	
5	10^{-5}	62	60	57	59.5	5.95 E+06	0.088
10	10^{-5}	30	42	26	32.0	3.20 E+06	0.358
20	10^{-4}	92	90	78	87.5	8.75 E+05	0.921
40	10^{-3}	40	41	32	38.5	3.85 E+05	2.309
60	10^{-1}	250	238	262	250.0	2.50 E+03	3.465

Table A-3. Data for UV inactivation of MS2 coliphage in DI water

Experiment Number[a]	Dose	Dilution	Count			Average count	CFU/mL	Log Inactivation
			1	2	3			
1	0	10^{-3}	211	196	179	195.0	1.95 E+05	
2		10^{-3}	179	178	176	178.0	1.78 E+05	
3		10^{-3}	176	179	178	178.0	1.78 E+05	
4		10^{-4}	176	163	141	142.0	1.42 E+06	
5		10^{-4}	94	96	95	94.5	9.45 E+05	
6		10^{-4}	124	117	112	118.0	1.18 E+05	
1	5	10^{-3}	105	104	102	104.8	1.95 E+05	0.275
2		10^{-3}	99	99	100	99.5	9.95 E+04	0.251
3		10^{-3}	121	106	91	106.3	1.06 E+03	0.224
4		10^{-4}	66	58	48	57.0	5.70 E+05	0.372
5		10^{-4}	56	48	39	47.5	4.75 E+05	0.296
6		10^{-4}	71	76	66	71.2	7.12 E+05	0.220
1	10	10^{-3}	46	43	48	45.5	4.55 E+04	0.632
2		10^{-3}	67	65	63	65.0	6.50 E+04	0.436
3		10^{-3}	96	87	77	86.5	8.68 E+04	0.312
4		10^{-4}	26	38	33	32.0	3.20 E+05	0.647
5		10^{-3}	244	216	272	244.0	2.44 E+05	0.588
6		10^{-4}	32	37	44	38.5	3.85 E+05	0.493
1	20	10^{-2}	55	54	52	53.5	5.35 E+03	1.562
2		10^{-2}	142	145	148	145.2	1.45 E+04	1.088
3		10^{-2}	246	239	234	240.3	2.40 E+04	0.869
4		10^{-3}	80	73	88	80.5	8.05 E+04	1.247
5		10^{-3}	53	60	66	59.6	5.96 E+04	1.200
6		10^{-3}	82	68	76	75.1	7.51 E+04	1.198
1	40	10^{-1}	170	189	150	170.1	1.70 E+03	2.061
2		10^{-1}	169	210	249	209.4	2.09 E+03	1.929
3		10^{-1}	172	177	184	177.9	1.78 E+03	1.999
4		10^{-2}	99	116	131	115.0	1.15 E+04	2.092
5		10^{-2}	105	94	122	108.0	1.08 E+04	1.942
6		10^{-2}	118	107	128	117.7	1.17 E+04	2.001
1	60	10^{0}	146	127	107	127.2	1.27 E+02	3.188
2		10^{0}	80	93	87	86.5	8.65 E+01	3.312
3		10^{0}	127	135	118	127.5	1.27 E+02	3.147
4		10^{-1}	209	187	194	198.0	1.98 E+03	2.856
5		10^{-1}	147	129	137	138.0	1.38 E+03	2.836
6		10^{-1}	157	131	109	133.2	1.13 E+03	2.946

[a] See Table 4-4.

APPENDIX B EXPERIMENTAL DATA AND INFORMATION FROM *B. subtilis*

Table B-1. Data for UV inactivation of *B. subtilis* spores for Preliminary Experiments (Sep 2006, North Saskatchewan River)

Dose	NTU[a]	Homo[b]	Dilution	Count			Average count	CFU/mL	Log Inactivation
				1	2	3			
0	Opt	Before	10^{-2}	102	95	99	98.67	9.87 E+03	
12	Opt	Before	10^{-2}	57	58	59	57.99	5.80 E+03	
12	Opt	Before	10^{-2}	68	65	62	64.95	6.50 E+03	0.193
12	Opt	Before	10^{-2}	62	67	71	66.56	6.66 E+03	
50	Opt	Before	10^{0}	48	37	43	42.42	4.24 E+01	
50	Opt	Before	10^{0}	34	36	38	35.96	3.60 E+01	2.387
50	Opt	Before	10^{0}	48	39	44	43.50	4.35 E+01	
0	Sub	Before	10^{-4}	59	55	57	56.97	5.70 E+05	
12	Sub	Before	10^{-4}	35	50	43	42.22	4.22 E+05	
12	Sub	Before	10^{-4}	43	35	27	34.38	3.44 E+05	0.165
12	Sub	Before	10^{-4}	42	37	40	39.61	3.96 E+05	
50	Sub	Before	10^{-1}	209	216	213	212.65	2.13 E+03	
50	Sub	Before	10^{-1}	183	184	184	183.67	1.84 E+03	2.435
50	Sub	Before	10^{-1}	241	234	227	233.93	2.34 E+03	
0	Poor	Before	10^{-4}	128	131	124	127.63	1.28 E+06	
12	Poor	Before	10^{-4}	78	84	90	83.86	8.39 E+05	
12	Poor	Before	10^{-4}	90	95	84	89.55	8.96 E+05	0.183
12	Poor	Before	10^{-4}	69	78	85	77.05	7.71 E+05	
50	Poor	Before	10^{-2}	59	56	52	55.59	5.56 E+03	
50	Poor	Before	10^{-2}	41	43	44	42.65	4.27 E+03	2.418
50	Poor	Before	10^{-2}	43	37	48	42.42	4.24 E+03	
0	Opt	After	10^{-2}	79	81	84	81.33	8.13 E+03	
12	Opt	After	10^{-2}	49	61	55	57.78	5.78 E+03	
12	Opt	After	10^{-2}	53	49	51	50.97	5.10 E+03	0.176
12	Opt	After	10^{-2}	65	57	49	56.62	5.62 E+03	
50	Opt	After	10^{0}	39	35	31	34.85	3.49 E+01	
50	Opt	After	10^{0}	31	29	27	28.95	2.90 E+01	2.400
50	Opt	After	10^{0}	30	34	37	33.54	3.35 E+01	
0	Sub	After	10^{-4}	50	47	44	46.94	4.69 E+05	
12	Sub	After	10^{-4}	29	28	30	28.99	2.90 E+05	
12	Sub	After	10^{-4}	44	40	35	39.49	3.95 E+05	0.157
12	Sub	After	10^{-4}	28	27	25	26.64	2.66 E+05	
50	Sub	After	10^{-1}	146	158	152	151.92	1.52 E+03	
50	Sub	After	10^{-1}	157	139	174	156.01	1.56 E+03	2.479
50	Sub	After	10^{-1}	155	160	165	159.94	1.60 E+03	
0	Poor	After	10^{-4}	90	82	86	85.94	8.59 E+05	
12	Poor	After	10^{-4}	60	62	57	59.63	5.96 E+05	
12	Poor	After	10^{-4}	56	54	51	53.63	5.36 E+05	0.173
12	Poor	After	10^{-4}	60	56	63	59.60	5.96 E+05	
50	Poor	After	10^{-2}	71	66	60	65.51	6.55 E+03	
50	Poor	After	10^{-2}	36	38	40	37.96	3.80 E+03	2.239
50	Poor	After	10^{-2}	34	42	50	41.49	4.15 E+03	

[a] NTU represented filter water conditions; optimal, sub-optimal and poor condition.

[b] Homogenization represented homogenization conditions; Homogenization before UV exposure and homogenization after UV exposure.

Table B-2. Data for UV inactivation of *B. subtilis* spores for Mid-Winter Experiments (Feb 2007, North Saskatchewan River)

Dose	NTU[a]	Homo[b]	Dilution	Count			Average count	CFU/mL	Log Inactivation
				1	2	3			
0	Opt	Before	10^{-2}	70	62	66	66.00	6.60 E+03	
15	Opt	Before	10^{-2}	25	31	28	28.00	2.80 E+03	
15	Opt	Before	10^{-2}	30	31	33	30.00	30.0 E+03	0.402
15	Opt	Before	10^{-2}	27	20	23	20.50	2.05 E+03	
40	Opt	Before	10^{0}	66	77	87	76.50	7.65 E+01	
40	Opt	Before	10^{0}	64	62	66	64.00	6.40 E+01	1.971
40	Opt	Before	10^{0}	69	74	72	71.50	7.15 E+01	
0	Sub	Before	10^{-3}	50	49	47	48.50	4.85 E+04	
15	Sub	Before	10^{-2}	233	217	249	233.00	2.33 E+04	
15	Sub	Before	10^{-2}	253	260	268	260.50	2.61 E+04	0.275
15	Sub	Before	10^{-2}	273	282	277	277.50	2.78 E+04	
40	Sub	Before	10^{-1}	120	117	114	117.00	1.17 E+03	
40	Sub	Before	10^{-1}	123	115	107	115.00	1.15 E+03	1.640
40	Sub	Before	10^{-1}	102	106	98	102.00	1.02 E+03	
0	Poor	Before	10^{-3}	333	344	355	344.00	3.44 E+05	
15	Poor	Before	10^{-3}	180	189	170	179.50	1.80 E+05	
15	Poor	Before	10^{-3}	176	224	200	200.00	2.00 E+05	0.237
15	Poor	Before	10^{-3}	217	220	213	216.50	2.17 E+05	
40	Poor	Before	10^{-2}	74	85	80	79.50	7.95 E+03	
40	Poor	Before	10^{-2}	83	84	82	83.00	8.30 E+03	1.632
40	Poor	Before	10^{-2}	65	79	92	78.50	7.85 E+03	
0	Opt	After	10^{-2}	72	77	82	77.00	7.70 E+03	
15	Opt	After	10^{-2}	36	36	35	35.50	3.55 E+03	
15	Opt	After	10^{-2}	34	27	29	39.00	3.90 E+03	0.409
15	Opt	After	10^{-2}	26	27	24	25.50	2.55 E+03	
40	Opt	After	10^{0}	132	124	128	128.00	1.28 E+02	
40	Opt	After	10^{0}	127	124	129	126.50	1.27 E+02	1.789
40	Opt	After	10^{0}	111	121	131	121.00	1.21 E+02	
0	Sub	After	10^{-3}	44	49	47	46.50	4.65 E+04	
15	Sub	After	10^{-2}	265	266	266	266.00	2.66 E+04	
15	Sub	After	10^{-2}	267	266	267	267.00	2.67 E+04	0.245
15	Sub	After	10^{-2}	261	255	267	261.00	2.61 E+04	
40	Sub	After	10^{-1}	130	157	144	143.50	1.44 E+03	
40	Sub	After	10^{-1}	140	142	137	139.50	1.40 E+03	1.518
40	Sub	After	10^{-1}	126	140	154	140.00	1.40 E+03	
0	Poor	After	10^{-3}	287	275	281	281.00	2.81 E+05	
15	Poor	After	10^{-3}	160	157	163	160.00	1.60 E+05	
15	Poor	After	10^{-3}	164	183	202	183.00	1.83 E+05	0.220
15	Poor	After	10^{-3}	164	173	154	163.50	1.64 E+05	
40	Poor	After	10^{-2}	67	104	86	85.50	8.55 E+03	
40	Poor	After	10^{-2}	85	85	85	85.00	8.50 E+03	1.495
40	Poor	After	10^{-2}	105	100	95	100.00	1.00 E+04	

[a] NTU represented filter water conditions; optimal, sub-optimal and poor condition.

[b] Homogenization represented homogenization conditions; Homogenization before UV exposure and homogenization after UV exposure.

Table B-3. Data for UV inactivation of *B. subtilis* spores for Mid-Winter Experiments (Mar 2007, Bow River)

Dose	NTU[a]	Homo[b]	Dilution	Count			Average count	CFU/mL	Log Inactivation
				1	2	3			
0	Opt	Before	10^{-2}	181	160	153	164.24	1.64 E+04	
15	Opt	Before	10^{-2}	53	45	49	49.00	4.90 E+03	
15	Opt	Before	10^{-2}	69	74	63	68.50	6.85 E+03	0.408
15	Opt	Before	10^{-2}	79	75	71	75.00	2.05 E+03	
40	Opt	Before	10^{0}	225	233	241	232.91	2.33 E+02	
40	Opt	Before	10^{0}	248	247	246	246.99	2.47 E+02	1.852
40	Opt	Before	10^{0}	189	224	231	213.85	2.14 E+02	
0	Sub	Before	10^{-4}	139	144	124	135.39	1.35 E+06	
15	Sub	Before	10^{-4}	63	74	69	68.50	6.85 E+05	
15	Sub	Before	10^{-4}	56	58	54	56.00	5.60 E+05	0.364
15	Sub	Before	10^{-4}	47	51	55	51.00	5.10 E+05	
40	Sub	Before	10^{-2}	237	266	251	251.50	2.52 E+03	
40	Sub	Before	10^{-2}	255	266	244	255.00	2.55 E+03	1.750
40	Sub	Before	10^{-2}	230	217	203	216.50	2.17 E+03	
0	Poor	Before	10^{-5}	34	37	38	36.29	3.63 E+06	
15	Poor	Before	10^{-4}	190	225	208	207.50	2.08 E+06	
15	Poor	Before	10^{-4}	186	199	173	186.00	1.86 E+06	0.283
15	Poor	Before	10^{-4}	173	173	172	172.50	1.73 E+06	
40	Poor	Before	10^{-3}	83	87	86	85.32	8.53 E+04	
40	Poor	Before	10^{-3}	102	91	95	95.89	9.59 E+04	1.608
40	Poor	Before	10^{-3}	89	86	87	87.32	8.73 E+04	
0	Opt	After	10^{-2}	259	256	270	261.60	2.62 E+04	
15	Opt	After	10^{-2}	120	116	118	118.00	1.18 E+04	
15	Opt	After	10^{-2}	91	93	94	92.50	9.25 E+03	0.407
15	Opt	After	10^{-2}	97	101	92	96.50	9.65 E+03	
40	Opt	After	10^{-1}	35	34	32	33.64	3.36 E+02	
40	Opt	After	10^{-1}	40	41	49	43.15	4.32 E+02	1.800
40	Opt	After	10^{-1}	45	49	52	48.58	4.86 E+02	
0	Sub	After	10^{-4}	94	89	114	98.43	9.84 E+05	
15	Sub	After	10^{-4}	50	47	49	48.50	4.85 E+05	
15	Sub	After	10^{-4}	45	54	35	44.50	4.45 E+05	0.385
15	Sub	After	10^{-4}	22	29	35	28.50	2.85 E+05	
40	Sub	After	10^{-2}	199	207	203	203.00	2.03 E+04	
40	Sub	After	10^{-2}	184	188	179	183.50	1.84 E+04	1.717
40	Sub	After	10^{-2}	168	181	193	180.50	1.81 E+04	
0	Poor	After	10^{-5}	39	31	34	34.51	3.45 E+06	
15	Poor	After	10^{-4}	177	156	154	162.00	1.62 E+06	
15	Poor	After	10^{-4}	171	191	187	182.79	1.83 E+06	0.293
15	Poor	After	10^{-4}	178	195	175	182.46	1.82 E+06	
40	Poor	After	10^{-3}	98	108	98	101.22	1.01 E+05	
40	Poor	After	10^{-3}	94	102	105	100.22	1.00 E+05	1.545
40	Poor	After	10^{-3}	85	97	101	94.08	9.41 E+05	

[a] NTU represented filter water conditions; optimal, sub-optimal and poor condition.
[b] Homogenization represented homogenization conditions; Homogenization before UV exposure and homogenization after UV exposure.

Table B-4. Data for UV inactivation of *B. subtilis* spores for Spring run-off Experiments (Mar 2007, Red Deer River)

Dose	NTU[a]	Homo[b]	Dilution	Count			Average count	CFU/mL	Log Inactivation
				1	2	3			
0	Opt	Before	10^{-2}	93	84	86	87.58	8.76 E+03	
15	Opt	Before	10^{-1}	209	234	238	226.62	2.27 E+03	0.569
15	Opt	Before	10^{-1}	277	242	238	251.74	2.52 E+03	
15	Opt	Before	10^{-1}	225	229	237	230.27	2.30 E+03	
40	Opt	Before	10^{0}	79	102	103	93.97	9.40 E+01	1.977
40	Opt	Before	10^{0}	79	105	92	91.38	9.14 E+01	
40	Opt	Before	10^{0}	84	87	105	91.55	9.16 E+01	
0	Sub	Before	10^{-3}	46	32	39	38.58	3.86 E+04	
15	Sub	Before	10^{-2}	125	114	103	113.65	1.14 E+04	0.499
15	Sub	Before	10^{-2}	118	114	112	114.64	1.15 E+04	
15	Sub	Before	10^{-2}	158	118	142	138.33	1.38 E+04	
40	Sub	Before	10^{-1}	52	55	51	52.64	5.26 E+02	1.857
40	Sub	Before	10^{-1}	57	48	56	53.50	5.35 E+02	
40	Sub	Before	10^{-1}	56	61	48	54.73	5.47 E+02	
0	Poor	Before	10^{-4}	65	51	53	56.00	5.60 E+05	
15	Poor	Before	10^{-4}	25	27	25	25.82	2.58 E+05	0.271
15	Poor	Before	10^{-4}	33	36	34	34.31	3.43 E+05	
15	Poor	Before	10^{-4}	28	25	37	29.59	2.96 E+05	
40	Poor	Before	10^{-2}	107	79	89	90.95	9.09 E+03	1.580
40	Poor	Before	10^{-2}	185	188	156	175.72	1.76 E+04	
40	Poor	Before	10^{-2}	204	168	203	190.90	1.90 E+04	
0	Opt	After	10^{-2}	115	88	96	99.04	9.90 E+03	
15	Opt	After	10^{-1}	262	261	274	265.60	2.66 E+03	0.532
15	Opt	After	10^{-1}	314	302	321	312.23	3.12 E+03	
15	Opt	After	10^{-1}	308	280	299	295.43	2.95 E+03	
40	Opt	After	10^{0}	263	217	216	231.00	2.31 E+02	1.831
40	Opt	After	10^{0}	114	131	104	115.80	1.16 E+02	
40	Opt	After	10^{0}	101	118	118	112.04	1.12 E+02	
0	Sub	After	10^{-3}	22	26	21	22.90	2.29 E+05	
15	Sub	After	10^{-2}	90	88	115	96.93	9.69 E+04	0.348
15	Sub	After	10^{-2}	85	110	102	98.43	9.84 E+04	
15	Sub	After	10^{-2}	117	110	112	112.96	1.13 E+04	
40	Sub	After	10^{-1}	69	75	56	66.18	6.62 E+02	1.557
40	Sub	After	10^{-1}	52	47	53	50.59	5.06 E+02	
40	Sub	After	10^{-1}	81	67	80	75.72	7.57 E+02	
0	Poor	After	10^{-4}	58	59	52	56.25	5.62 E+05	
15	Poor	After	10^{-4}	23	21	22	22.03	2.20 E+05	0.306
15	Poor	After	10^{-4}	39	32	40	36.82	3.68 E+05	
15	Poor	After	10^{-4}	24	17	31	23.30	2.33 E+05	
40	Poor	After	10^{-2}	186	190	174	183.21	1.83 E+04	1.483
40	Poor	After	10^{-2}	192	184	194	189.95	1.89 E+04	
40	Poor	After	10^{-2}	174	195	176	181.42	1.81 E+04	

[a] NTU represented filter water conditions; optimal, sub-optimal and poor condition.

[b] Homogenization represented homogenization conditions; Homogenization before UV exposure and homogenization after UV exposure.

Table B-5. Data for UV inactivation of *B. subtilis* spores for Spring run-off Experiments (April 2007, North Saskatchewan River)

Dose	NTU[a]	Homo[b]	Dilution	Count			Average count	CFU/mL	Log Inactivation
				1	2	3			
0	Opt	Before	10^{-2}	35	73	29	45.67	4.57 E+03	
15	Opt	Before	10^{-1}	89	91	109	95.93	9.59 E+02	
15	Opt	Before	10^{-1}	92	100	84	91.77	9.18 E+02	0.715
15	Opt	Before	10^{-1}	81	72	78	76.91	7.69 E+02	
40	Opt	Before	10^{0}	50	43	49	47.23	4.72 E+01	
40	Opt	Before	10^{0}	45	54	48	48.86	4.89 E+01	1.969
40	Opt	Before	10^{0}	54	47	53	51.24	5.12 E+01	
0	Sub	Before	10^{-3}	92	90	88	89.99	8.99 E+04	
15	Sub	Before	10^{-3}	49	50	54	50.95	5.95 E+04	
15	Sub	Before	10^{-3}	52	56	59	55.59	5.56 E+04	0.205
15	Sub	Before	10^{-3}	60	56	69	61.43	6.14 E+04	
40	Sub	Before	10^{-1}	264	269	239	256.99	2.57 E+03	
40	Sub	Before	10^{-1}	246	287	303	277.60	2.77 E+03	1.516
40	Sub	Before	10^{-1}	291	276	301	289.91	2.89 E+03	
0	Poor	Before	10^{-4}	83	84	86	84.32	8.43 E+05	
15	Poor	Before	10^{-4}	44	47	42	44.29	4.43 E+05	
15	Poor	Before	10^{-4}	53	44	55	50.43	5.04 E+05	0.217
15	Poor	Before	10^{-4}	67	50	58	57.92	5.79 E+05	
40	Poor	Before	10^{-2}	231	220	244	231.46	2.31 E+04	
40	Poor	Before	10^{-2}	292	271	252	271.16	2.71 E+04	1.527
40	Poor	Before	10^{-2}	272	291	268	276.82	2.76 E+04	
0	Opt	After	10^{-2}	58	36	51	48.33	4.83 E+03	
15	Opt	After	10^{-1}	180	176	191	182.22	1.82 E+03	
15	Opt	After	10^{-1}	86	76	80	80.56	8.06 E+02	0.633
15	Opt	After	10^{-1}	89	71	87	81.92	8.19 E+02	
40	Opt	After	10^{0}	68	70	65	67.64	6.76 E+01	
40	Opt	After	10^{0}	53	79	68	65.79	6.58 E+01	1.864
40	Opt	After	10^{0}	69	71	56	64.98	6.50 E+01	
0	Sub	After	10^{-3}	76	74	78	75.98	7.60 E+04	
15	Sub	After	10^{-3}	44	38	31	37.28	3.72 E+04	
15	Sub	After	10^{-3}	59	47	48	51.06	5.10 E+04	0.230
15	Sub	After	10^{-3}	48	44	43	44.95	4.49 E+04	
40	Sub	After	10^{-1}	222	202	187	203.16	2.03 E+03	
40	Sub	After	10^{-1}	223	286	231	245.15	2.45 E+03	1.518
40	Sub	After	10^{-1}	252	247	238	245.60	2.46 E+03	
0	Poor	After	10^{-4}	61	58	67	61.89	6.19 E+05	
15	Poor	After	10^{-4}	28	36	30	31.15	3.12 E+05	
15	Poor	After	10^{-4}	37	26	23	28.07	2.81 E+05	0.287
15	Poor	After	10^{-4}	44	32	34	36.31	3.63 E+05	
40	Poor	After	10^{-2}	207	231	195	210.48	2.10 E+04	
40	Poor	After	10^{-2}	269	261	275	268.27	2.68 E+04	1.415
40	Poor	After	10^{-2}	276	268	263	268.95	2.69 E+04	

[a] NTU represented filter water conditions; optimal, sub-optimal and poor condition.
[b] Homogenization represented homogenization conditions; Homogenization before UV exposure and homogenization after UV exposure.

APPENDIX C EXPERIMENTAL DATA AND INFORMATION FROM MS2 coliphage

Table C-1. Data for UV inactivation of MS2 coliphage for Preliminary Experiments (Sep 2006, North Saskatchewan River)

Dose	NTU[a]	Blend[b]	Dilution	Count			Average count	CFU/mL	Log Inactivation
				1	2	3			
0	Opt	Before	10^{-2}	125	121	129	125.00	1.25 E+04	
15	Opt	Before	10^{-1}	180	152	182	170.76	1.71 E+03	
15	Opt	Before	10^{-1}	158	195	175	175.35	1.75 E+03	0.863
15	Opt	Before	10^{-1}	185	146	176	168.14	1.68 E+03	
40	Opt	Before	10^{0}	105	94	98	98.89	9.89 E+01	
40	Opt	Before	10^{0}	109	91	93	97.35	9.73 E+01	2.104
40	Opt	Before	10^{0}	103	94	99	98.60	9.86 E+01	
0	Sub	Before	10^{-4}	62	67	71	66.56	6.66 E+05	
15	Sub	Before	10^{-3}	90	98	102	96.54	9.65 E+04	
15	Sub	Before	10^{-3}	112	115	108	111.63	1.11 E+05	0.808
15	Sub	Before	10^{-3}	92	107	110	102.69	1.03 E+05	
40	Sub	Before	10^{-2}	101	111	105	105.59	1.06 E+04	
40	Sub	Before	10^{-2}	111	96	104	103.48	1.03 E+04	1.819
40	Sub	Before	10^{-2}	102	83	99	94.28	9.42 E+04	
0	Poor	Before	10^{-3}	178	175	176	176.33	1.76 E+06	
15	Poor	Before	10^{-3}	29	28	30	28.99	2.90 E+05	
15	Poor	Before	10^{-3}	29	35	41	34.65	3.47 E+05	0.769
15	Poor	Before	10^{-3}	21	25	36	26.64	2.66 E+05	
40	Poor	Before	10^{-2}	35	46	29	36.01	3.60 E+03	
40	Poor	Before	10^{-2}	31	30	31	30.66	3.07 E+03	1.724
40	Poor	Before	10^{-2}	42	46	49	45.58	4.56 E+03	
0	Opt	After	10^{-2}	115	102	108	108.33	1.08 E+03	
15	Opt	After	10^{-1}	168	172	165	168.31	1.68 E+03	
15	Opt	After	10^{-1}	180	176	170	175.29	1.75 E+03	0.803
15	Opt	After	10^{-1}	170	171	162	167.62	1.68 E+03	
40	Opt	After	10^{0}	179	161	171	170.17	1.70 E+01	
40	Opt	After	10^{0}	169	164	174	168.95	1.69 E+01	1.798
40	Opt	After	10^{0}	171	194	171	178.35	1.78 E+01	
0	Sub	After	10^{-4}	52	55	64	56.78	5.68 E+05	
15	Sub	After	10^{-3}	96	95	105	98.57	9.86 E+04	
15	Sub	After	10^{-3}	81	85	92	85.88	8.59 E+04	0.777
15	Sub	After	10^{-3}	99	105	98	100.62	1.01 E+05	
40	Sub	After	10^{-2}	122	119	105	115.09	1.15 E+04	
40	Sub	After	10^{-2}	111	117	121	116.26	1.16 E+04	1.690
40	Sub	After	10^{-2}	131	97	125	116.68	1.17 E+04	
0	Poor	After	10^{-3}	128	122	125	124.98	1.25 E+05	
15	Poor	After	10^{-3}	19	21	27	22.09	2.21 E+04	
15	Poor	After	10^{-3}	28	29	19	24.89	2.49 E+04	0.747
15	Poor	After	10^{-3}	21	22	18	20.26	2.02 E+04	
40	Poor	After	10^{-2}	45	32	37	37.63	3.76 E+03	
40	Poor	After	10^{-2}	36	31	47	37.43	3.74 E+03	1.522
40	Poor	After	10^{-2}	33	36	41	36.52	3.65 E+03	

[a] NTU represented filter water conditions; optimal, sub-optimal and poor condition.

[b] Blend represented Blended conditions; Blended before UV exposure and Blended after UV exposure.

Table C-2. Data for UV inactivation of MS2 coliphage for Mid-Winter Experiments (Feb 2007, North Saskatchewan River)

Dose	NTU[a]	Blend[b]	Dilution	Count			Average count	CFU/mL	Log Inactivation
				1	2	3			
0	Opt	Before	10^{-2}	96	123	114	110.41	1.10 E+04	
15	Opt	Before	10^{-1}	63	81	72	71.62	7.16 E+02	
15	Opt	Before	10^{-1}	46	41	45	43.95	4.40 E+02	1.295
15	Opt	Before	10^{-1}	58	51	55	54.59	5.46 E+02	
40	Opt	Before	10^{0}	61	58	59	59.32	5.93 E+01	
40	Opt	Before	10^{0}	51	52	55	52.64	5.26 E+01	2.311
40	Opt	Before	10^{0}	47	54	50	50.25	5.03 E+01	
0	Sub	Before	10^{-4}	32	41	38	36.80	3.68 E+05	
15	Sub	Before	10^{-3}	27	37	33	32.06	3.21 E+04	
15	Sub	Before	10^{-3}	41	40	45	41.95	4.19 E+04	1.006
15	Sub	Before	10^{-3}	33	34	39	35.24	3.52 E+04	
40	Sub	Before	10^{-2}	35	38	37	36.65	3.66 E+03	
40	Sub	Before	10^{-2}	31	30	33	31.31	3.13 E+03	2.059
40	Sub	Before	10^{-2}	24	31	32	28.77	2.87 E+03	
0	Poor	Before	10^{-3}	182	102	157	142.32	1.42 E+05	
15	Poor	Before	10^{-3}	25	22	23	23.30	2.33 E+04	
15	Poor	Before	10^{-3}	19	21	25	21.53	2.15 E+04	0.795
15	Poor	Before	10^{-3}	25	22	24	23.63	2.36 E+04	
40	Poor	Before	10^{-2}	23	25	24	23.98	2.39 E+03	
40	Poor	Before	10^{-2}	26	23	25	24.63	2.46 E+03	1.757
40	Poor	Before	10^{-2}	30	22	27	26.12	2.61 E+03	
0	Opt	After	10^{-2}	92	94	102	95.90	9.59 E+03	
15	Opt	After	10^{-1}	162	142	148	150.04	1.50 E+03	
15	Opt	After	10^{-1}	152	131	150	144.01	1.44 E+03	0.808
15	Opt	After	10^{-1}	132	162	169	153.46	1.53 E+03	
40	Opt	After	10^{0}	162	149	163	157.87	1.57 E+02	
40	Opt	After	10^{0}	165	175	155	164.80	1.64 E+02	1.780
40	Opt	After	10^{0}	152	164	150	155.21	1.55 E+02	
0	Sub	After	10^{-4}	74	71	72	72.32	7.23 E+05	
15	Sub	After	10^{-3}	126	142	139	135.49	1.35 E+05	
15	Sub	After	10^{-3}	119	131	128	125.90	1.25 E+05	0.727
15	Sub	After	10^{-3}	151	148	139	145.91	1.45 E+05	
40	Sub	After	10^{-2}	163	156	165	161.29	1.61 E+04	
40	Sub	After	10^{-2}	149	161	154	154.59	1.54 E+04	1.657
40	Sub	After	10^{-2}	178	158	152	162.30	1.62 E+04	
0	Poor	After	10^{-3}	105	104	102	103.66	1.04 E+05	
15	Poor	After	10^{-3}	21	20	24	21.60	2.16 E+04	
15	Poor	After	10^{-3}	18	21	20	19.63	1.96 E+04	0.697
15	Poor	After	10^{-3}	21	20	23	21.29	2.13 E+04	
40	Poor	After	10^{-2}	22	31	25	25.74	2.57 E+03	
40	Poor	After	10^{-2}	28	33	22	27.29	2.73 E+03	1.595
40	Poor	After	10^{-2}	19	25	37	26.00	2.60 E+03	

[a] NTU represented filter water conditions; optimal, sub-optimal and poor condition.
[b] Blend represented Blended conditions; Blended before UV exposure and Blended after UV exposure.

Table C-3. Data for UV inactivation of MS2 coliphage for Mid-Winter Experiments (Mar 2007, Bow River)

Dose	NTU[a]	Blend[b]	Dilution	Count			Average count	CFU/mL	Log Inactivation
				1	2	3			
0	Opt	Before	10^{-2}	109	112	111	110.50	1.11 E+04	
15	Opt	Before	10^{-2}	124	126	122	124.00	1.24 E+03	0.979
15	Opt	Before	10^{-2}	128	115	101	114.50	1.15 E+03	
15	Opt	Before	10^{-2}	111	110	110	110.00	1.10 E+03	
40	Opt	Before	10^{0}	100	106	103	103.00	1.03 E+01	2.082
40	Opt	Before	10^{0}	87	79	94	86.50	8.65 E+01	
40	Opt	Before	10^{0}	94	86	78	86.00	8.60 E+01	
0	Sub	Before	10^{-4}	57	53	55	55.00	5.50 E+05	
15	Sub	Before	10^{-3}	70	73	72	71.50	7.15 E+04	0.854
15	Sub	Before	10^{-3}	83	82	84	83.00	8.30 E+04	
15	Sub	Before	10^{-3}	74	77	79	76.50	7.65 E+04	
40	Sub	Before	10^{-2}	78	83	81	80.50	8.05 E+03	1.830
40	Sub	Before	10^{-2}	80	74	85	79.50	7.95 E+03	
40	Sub	Before	10^{-2}	87	84	81	84.00	8.40 E+03	
0	Poor	Before	10^{-4}	86	83	75	81.33	8.13 E+05	
15	Poor	Before	10^{-3}	126	120	123	123.00	1.23 E+04	0.785
15	Poor	Before	10^{-3}	129	126	131	128.50	1.29 E+04	
15	Poor	Before	10^{-3}	147	149	151	149.00	1.49 E+04	
40	Poor	Before	10^{-2}	134	139	137	136.50	1.37 E+03	1.746
40	Poor	Before	10^{-2}	159	168	149	158.50	1.59 E+03	
40	Poor	Before	10^{-2}	140	144	148	144.00	1.44 E+03	
0	Opt	After	10^{-2}	74	71	73	72.50	7.25 E+03	
15	Opt	After	10^{-1}	82	86	77	81.50	8.15 E+02	0.924
15	Opt	After	10^{-1}	90	91	85	87.50	8.75 E+02	
15	Opt	After	10^{-1}	76	105	91	90.50	9.05 E+02	
40	Opt	After	10^{0}	68	67	69	68.00	6.80 E+01	2.053
40	Opt	After	10^{0}	60	62	63	61.50	6.15 E+01	
40	Opt	After	10^{0}	62	64	62	63.00	6.30 E+01	
0	Sub	After	10^{-3}	72	91	82	81.50	8.15 E+04	
15	Sub	After	10^{-2}	120	112	128	120.00	1.20 E+04	0.821
15	Sub	After	10^{-2}	130	117	104	117.00	1.17 E+04	
15	Sub	After	10^{-2}	135	130	133	132.50	1.32 E+04	
40	Sub	After	10^{-1}	148	147	148	147.50	1.47 E+03	1.733
40	Sub	After	10^{-1}	159	152	145	152.00	1.52 E+03	
40	Sub	After	10^{-1}	161	144	153	152.50	1.52 E+03	
0	Poor	After	10^{-3}	118	121	120	119.50	1.19 E+05	
15	Poor	After	10^{-2}	219	226	211	218.50	2.18 E+04	0.753
15	Poor	After	10^{-2}	199	206	212	205.50	2.06 E+04	
15	Poor	After	10^{-2}	210	208	209	209.00	2.09 E+04	
40	Poor	After	10^{-1}	253	248	257	252.50	2.52 E+03	1.683
40	Poor	After	10^{-1}	233	247	260	246.50	2.46 E+03	
40	Poor	After	10^{-1}	257	232	245	244.50	2.44 E+03	

[a] NTU represented filter water conditions; optimal, sub-optimal and poor condition.

[b] Blend represented Blended conditions; Blended before UV exposure and Blended after UV exposure.

Table C-4. Data for UV inactivation of MS2 coliphage for Spring run-off Experiments (Mar 2007, Red Deer River)

Dose	NTU[a]	Blend[b]	Dilution	Count			Average count	CFU/mL	Log Inactivation
				1	2	3			
0	Opt	Before	10^{-1}	79	92	85	85.25	8.52 E+02	
15	Opt	Before	10^{0}	85	100	89	91.12	9.11 E+01	
15	Opt	Before	10^{0}	83	86	105	90.84	9.08 E+01	0.951
15	Opt	Before	10^{0}	101	111	103	104.91	1.05 E+02	
40	Opt	Before	10^{0}	9	2	6	4.76	4.76 E+00	
40	Opt	Before	10^{0}	8	5	6	6.21	6.21 E+00	2.136
40	Opt	Before	10^{0}	6	11	8	8.08	8.08 E+00	
0	Sub	Before	10^{-2}	68	77	77	73.87	7.38 E+03	
15	Sub	Before	10^{-1}	100	86	86	90.43	9.04 E+02	
15	Sub	Before	10^{-1}	99	98	105	100.62	1.00 E+03	0.882
15	Sub	Before	10^{-1}	103	99	97	99.64	9.96 E+02	
40	Sub	Before	10^{0}	69	65	63	65.62	6.56 E+01	
40	Sub	Before	10^{0}	60	68	68	65.22	6.52 E+01	2.044
40	Sub	Before	10^{0}	65	73	71	69.58	6.95 E+01	
0	Poor	Before	10^{-3}	72	65	58	64.75	6.47 E+04	
15	Poor	Before	10^{-2}	75	92	73	79.57	7.95 E+03	
15	Poor	Before	10^{-2}	94	95	89	92.63	9.26 E+03	0.856
15	Poor	Before	10^{-2}	89	106	103	99.04	9.90 E+03	
40	Poor	Before	10^{-1}	84	84	100	89.03	8.90 E+02	
40	Poor	Before	10^{-1}	116	100	116	110.04	1.10 E+03	1.815
40	Poor	Before	10^{-1}	99	89	109	98.66	9.86 E+02	
0	Opt	After	10^{-1}	60	58	59	58.99	5.89 E+03	
15	Opt	After	10^{0}	55	71	56	60.25	6.02 E+03	
15	Opt	After	10^{0}	64	54	60	59.19	5.91 E+03	0.994
15	Opt	After	10^{0}	64	61	55	59.88	5.98 E+03	
40	Opt	After	10^{0}	7	9	5	6.80	6.80 E+01	
40	Opt	After	10^{0}	9	5	9	7.40	7.40 E+01	1.896
40	Opt	After	10^{0}	12	8	6	8.32	8.32 E+01	
0	Sub	After	10^{-1}	161	182	171	171.18	1.71 E+03	
15	Sub	After	10^{-1}	37	40	34	36.88	3.68 E+02	
15	Sub	After	10^{-1}	25	23	24	24.49	2.44 E+02	0.755
15	Sub	After	10^{-1}	32	27	29	29.93	2.99 E+02	
40	Sub	After	10^{0}	35	72	32	43.20	4.32 E+01	
40	Sub	After	10^{0}	29	24	23	25.20	2.52 E+01	1.778
40	Sub	After	10^{0}	19	24	19	20.54	2.05 E+01	
0	Poor	After	10^{-2}	200	207	203	203.47	2.03 E+04	
15	Poor	After	10^{-2}	34	32	37	34.41	3.44 E+03	
15	Poor	After	10^{-2}	45	38	33	38.54	3.85 E+03	0.717
15	Poor	After	10^{-2}	47	42	44	44.43	4.44 E+03	
40	Poor	After	10^{-1}	65	65	67	65.99	6.59 E+02	
40	Poor	After	10^{-1}	75	77	80	77.46	7.74 E+02	1.454
40	Poor	After	10^{-1}	69	74	71	71.46	7.15 E+02	

[a] NTU represented filter water conditions; optimal, sub-optimal and poor condition.

[b] Blend represented Blended conditions; Blended before UV exposure and Blended after UV exposure.

Table C-5. Data for UV inactivation of MS2 coliphage for Spring run-off Experiments (April 2007, North Saskatchewan River)

Dose	NTU[a]	Blend[b]	Dilution	Count			Average count	CFU/mL	Log Inactivation
				1	2	3			
0	Opt	Before	10^{-1}	126	129	124	126.31	1.26 E+03	
15	Opt	Before	10^{0}	125	132	129	128.63	1.28 E+02	
15	Opt	Before	10^{0}	133	126	127	128.63	1.28 E+02	0.985
15	Opt	Before	10^{0}	128	146	132	135.11	1.35 E+02	
40	Opt	Before	10^{0}	9	12	9	9.91	9.91 E+00	
40	Opt	Before	10^{0}	7	11	8	8.51	8.51 E+00	2.147
40	Opt	Before	10^{0}	6	9	12	8.65	8.65 E+00	
0	Sub	Before	10^{-2}	156	152	144	150.58	1.50 E+04	
15	Sub	Before	10^{-1}	182	172	175	176.28	1.76 E+03	
15	Sub	Before	10^{-1}	198	218	189	201.30	2.01 E+03	0.909
15	Sub	Before	10^{-1}	175	189	175	179.54	1.79 E+03	
40	Sub	Before	10^{0}	136	137	128	133.60	1.33 E+02	
40	Sub	Before	10^{0}	122	131	140	130.79	1.30 E+02	2.044
40	Sub	Before	10^{0}	137	146	151	144.54	1.44 E+02	
0	Poor	Before	10^{-3}	77	61	71	69.34	6.93 E+04	
15	Poor	Before	10^{-2}	88	100	91	92.86	9.28 E+03	
15	Poor	Before	10^{-2}	105	100	97	100.61	1.00 E+04	0.844
15	Poor	Before	10^{-2}	100	109	105	104.60	1.04 E+04	
40	Poor	Before	10^{-1}	104	105	115	107.89	1.07 E+03	
40	Poor	Before	10^{-1}	125	119	130	124.59	1.24 E+03	1.777
40	Poor	Before	10^{-1}	142	118	127	128.62	1.28 E+03	
0	Opt	After	10^{-1}	116	121	119	118.65	1.18 E+03	
15	Opt	After	10^{0}	135	162	138	144.51	1.44 E+02	
15	Opt	After	10^{0}	112	155	148	136.96	1.36 E+02	0.928
15	Opt	After	10^{0}	157	148	115	138.77	1.38 E+02	
40	Opt	After	10^{0}	16	12	17	14.83	1.48 E+01	
40	Opt	After	10^{0}	26	20	17	20.68	2.06 E+01	1.823
40	Opt	After	10^{0}	16	24	16	18.32	1.83 E+01	
0	Sub	After	10^{-2}	148	151	144	147.63	1.47 E+04	
15	Sub	After	10^{-1}	207	197	190	197.87	1.97 E+03	
15	Sub	After	10^{-1}	220	224	219	220.09	2.20 E+03	0.844
15	Sub	After	10^{-1}	233	216	201	216.27	2.16 E+03	
40	Sub	After	10^{0}	261	256	247	254.60	2.54 E+02	
40	Sub	After	10^{0}	250	241	215	234.85	2.34 E+02	1.780
40	Sub	After	10^{0}	264	257	221	246.59	2.46 E+02	
0	Poor	After	10^{-3}	77	61	71	69.34	6.93 E+04	
15	Poor	After	10^{-2}	131	142	137	136.59	1.36 E+04	
15	Poor	After	10^{-2}	147	154	124	141.06	1.41 E+04	0.676
15	Poor	After	10^{-2}	164	158	161	160.98	1.60 E+04	
40	Poor	After	10^{-1}	227	208	215	216.52	2.16 E+03	
40	Poor	After	10^{-1}	225	200	198	207.31	2.07 E+03	1.510
40	Poor	After	10^{-1}	197	257	208	219.90	2.19 E+03	

[a] NTU represented filter water conditions; optimal, sub-optimal and poor condition.

[b] Blend represented Blended conditions; Blended before UV exposure and Blended after UV exposure.

APPENDIX D STATISTICAL ANALYSIS

Table D-1. Summary output of ANOVA for Preliminary Experiments (Sep 2006, North Saskatchewan River) for *B. subtilis*

Anova: Three-Factor With Replication

Source	SS	df	MS	F-ratio	*p*-value
NTU(A)[a]	0.015	2	0.070	2.188	0.134
Homogenization(B)[b]	0.050	1	0.050	1.418	0.245
UV(C)[c]	44.398	1	44.398	13036.871	1.055×10^{-11}
A x B	0.023	2	0.012	3.384	0.051
A x C	0.020	2	0.010	2.944	0.072
B x C	0.002	1	0.002	0.710	0.408
A x B x C	0.020	2	0.010	2.874	0.076
Error	0.082	24	0.003		

[a] NTU represented filter water conditions; optimal, sub-optimal and poor condition.
[b] Homogenization represented homogenization conditions; Homogenization before UV exposure and homogenization after UV exposure.
[c] UV represented UV dose conditions; 12 mJ/cm^2 and 50 mJ/cm^2.

Table D-2. Summary output of ANOVA for Mid-Winter Condition Experiment (Feb 2007, North Saskatchewan River) for *B. subtilis*

Anova: Three-Factor With Replication

Source	SS	df	MS	F-ratio	*p*-value
NTU(A)[a]	0.452	2	0.226	124.826	1.465×10^{-11}
Homogenization(B)[b]	0.059	1	0.059	32.483	7.168×10^{-6}
UV(C)[c]	16.978	1	16.978	9379.410	1.093×10^{-11}
A x B	0.001	2	0.001	0.068	0.934
A x C	0.042	2	0.021	11.618	2.959×10^{-4}
B x C	0.039	1	0.039	21.749	9.768×10^{-5}
A x B x C	0.004	2	0.002	10.660	0.360
Error	0.043	24	0.002		

[a] NTU represented filter water conditions; optimal, sub-optimal and poor condition.
[b] Homogenization represented homogenization conditions; Homogenization before UV exposure and homogenization after UV exposure.
[c] UV represented UV dose conditions; 15 mJ/cm^2 and 40 mJ/cm^2.

Table D-3. Summary output of ANOVA for mid-winter Condition Experiment (Mar 2007, Bow River) for *B. subtilis*

Anova: Three-Factor With Replication

Source	SS	df	MS	F-ratio	*p*-value
NTU(A)[a]	0.215	2	0.107	28.790	4.200×10^{-7}
Homogenization(B)[b]	0.014	1	0.014	3.949	0.003
UV(C)[c]	16.413	1	16.413	4390.260	1.192×10^{-11}
A x B	0.001	2	0.001	0.189	0.828
A x C	0.023	2	0.016	3.155	0.060
B x C	0.008	1	0.008	2.254	0.146
A x B x C	0.001	2	0.001	0.026	0.973
Error	0.089	24	0.003		

[a] NTU represented filter water conditions; optimal, sub-optimal and poor condition.
[b] Homogenization represented homogenization conditions; Homogenization before UV exposure and homogenization after UV exposure.
[c] UV represented UV dose conditions; 15 mJ/cm^2 and 40 mJ/cm^2.

Table D-4. Summary output of ANOVA for spring run-off Condition Experiment (Mar 2007, Red Deer River) for *B. subtilis*

Anova: Three-Factor With Replication

Source	SS	df	MS	F-ratio	*p*-value
NTU(A)[a]	0.583	2	0.291	36.802	4.900×10^{-8}
Homogenization(B)[b]	0.116	1	0.116	14.667	8.093×10^{-4}
UV(C)[c]	15.004	1	15.004	1894.220	1.325×10^{-11}
A x B	0.061	2	0.031	3.899	0.034
A x C	0.022	2	0.011	1.406	0.264
B x C	0.040	1	0.040	5.109	0.033
A x B x C	0.001	2	0.001	0.067	0.935
Error	0.190	24	0.007		

[a] NTU represented filter water conditions; optimal, sub-optimal and poor condition.
[b] Homogenization represented homogenization conditions; Homogenization before UV exposure and homogenization after UV exposure.
[c] UV represented UV dose conditions; 15 mJ/cm^2 and 40 mJ/cm^2.

Table D-5. Summary output of ANOVA for spring run-off Condition Experiment (April 2007, North Saskatchewan River) for *B. subtilis*

Anova: Three-Factor With Replication

Source	SS	df	MS	F-ratio	*p*-value
NTU(A)[a]	1.544	2	0.772	139.875	1.431×10^{-11}
Homogenization(B)[b]	0.070	1	0.070	13.712	2.530×10^{-3}
UV(C)[c]	13.882	1	13.882	2514.010	1.277×10^{-11}
A x B	0.014	2	0.007	1.310	0.288
A x C	0.013	2	0.006	1.265	0.300
B x C	0.017	1	0.017	3.080	0.092
A x B x C	0.011	2	0.006	1.045	0.367
Error	0.132	24	0.005		

[a] NTU represented filter water conditions; optimal, sub-optimal and poor condition.
[b] Homogenization represented homogenization conditions; Homogenization before UV exposure and homogenization after UV exposure.
[c] UV represented UV dose conditions; 15 mJ/cm^2 and 40 mJ/cm^2.

Table D-6. Summary output of ANOVA for Preliminary Experiments (Sep 2006, North Saskatchewan River) for MS2 coliphage

Anova: Three-Factor With Replication

Source	SS	Df	MS	F-ratio	*p*-value
NTU(A)[a]	0.268	2	0.134	98.190	1.541×10^{-11}
Blender(B)[b]	0.123	1	0.123	90.226	1.33×10^{-9}
UV(C)[c]	8.544	1	8.544	6240.070	1.144×10^{-11}
A x B	0.019	2	0.010	7.187	0.035
A x C	0.113	2	0.056	4.159	1.600×10^{-8}
B x C	0.056	1	0.056	4.119	1.230×10^{-6}
A x B x C	0.009	2	0.009	3.376	0.051
Error	0.032	24	0.001		

[a] NTU represented filter water conditions; optimal, sub-optimal and poor condition.
[b] Blender represented Blended conditions; Blended before UV exposure and Blended after UV exposure.
[c] UV represented UV dose conditions; 12 mJ/cm^2 and 50 mJ/cm^2.

Table D-7 Summary output of ANOVA for Mid-Winter Condition Experiment (Feb 2007, North Saskatchewan River) for MS2 coliphage

Anova: Three-Factor With Replication

Source	SS	df	MS	F-ratio	*p*-value
NTU(A)[a]	0.931	2	0.465	190.638	1.346×10^{-11}
Blender (B)[b]	0.768	1	0.768	314.547	1.738×10^{-11}
UV(C)[c]	8.100	1	8.100	3314.750	1.233×10^{-11}
A x B	0.124	2	0.062	25.447	1.172×10^{-6}
A x C	0.008	2	0.004	1.652	0.212
B x C	0.032	1	0.032	13.428	0.001
A x B x C	0.004	2	0.002	0.963	0.396
Error	0.058	24	0.002		

[a] NTU represented filter water conditions; optimal, sub-optimal and poor condition.
[b] Blender represented Blended conditions; Blended before UV exposure and Blended after UV exposure.
[c] UV represented UV dose conditions; 15 mJ/cm^2 and 40 mJ/cm^2.

Table D-8. Summary output of ANOVA for mid-winter Condition Experiment (Mar 2007, Bow River) for MS2 coliphage

Anova: Three-Factor With Replication

Source	SS	df	MS	F-ratio	*p*-value
NTU(A)[a]	0.463	2	0.231	306.995	1.23×10^{-11}
Blender (B)[b]	0.023	1	0.023	31.749	8.420×10^{-6}
UV(C)[c]	9.028	1	9.028	1195.470	1.065×10^{-11}
A x B	0.001	2	0.001	0.582	0.566
A x C	0.001	2	0.001	39.221	2.700×10^{-8}
B x C	0.059	1	0.059	1.452	0.239
A x B x C	0.001	2	0.001	2.076	0.147
Error	0.018	24	0.001		

[a] NTU represented filter water conditions; optimal, sub-optimal and poor condition.
[b] Blender represented Blended conditions; Blended before UV exposure and Blended after UV exposure.
[c] UV represented UV dose conditions; 15 mJ/cm^2 and 40 mJ/cm^2.

Table D-9 Summary output of ANOVA for spring run-off Condition Experiment (Mar 2007, Red Deer River) for MS2 coliphage

Anova: Three-Factor With Replication

Source	SS	df	MS	F-ratio	*p*-value
NTU(A)[a]	0.484	2	0.242	46.898	5.120×10^{-9}
Blender (B)[b]	0.292	1	0.292	56.671	9.100×10^{-8}
UV(C)[c]	8.909	1	8.909	1725.840	1.341×10^{-11}
A x B	0.034	2	0.017	3.376	0.051
A x C	0.101	2	0.055	9.838	0.001
B x C	0.103	1	0.103	20.045	0.001
A x B x C	0.008	2	0.004	0.805	0.458
Error	0.123	24	0.005		

[a] NTU represented filter water conditions; optimal, sub-optimal and poor condition.
[b] Blender represented Blended conditions; Blended before UV exposure and Blended after UV exposure.
[c] UV represented UV dose conditions; 15 mJ/cm^2 and 40 mJ/cm^2.

Table D-10. Summary output of ANOVA for spring run-off Condition Experiment (April 2007, North Saskatchewan River) for MS2 coliphage

Anova: Three-Factor With Replication

Source	SS	df	MS	F-ratio	*p*-value
NTU(A)[a]	0.474	2	0.237	213.728	1.318×10^{-11}
Blender (B)[b]	0.318	1	0.318	286.600	1.767×10^{-3}
UV(C)[c]	8.639	1	8.639	7776.350	1.116×10^{-11}
A x B	0.002	2	0.001	1.384	0.278
A x C	0.049	2	0.024	22.177	3.509×10^{-6}
B x C	0.075	1	0.075	67.726	1.900×10^{-8}
A x B x C	0.012	2	0.006	5.661	0.009
Error	0.026	24	0.001		

[a] NTU represented filter water conditions; optimal, sub-optimal and poor condition.
[b] Blender represented Blended conditions; Blended before UV exposure and Blended after UV exposure.
[c] UV represented UV dose conditions; 15 mJ/cm^2 and 40 mJ/cm^2.

Table D-11. Summary output of ANOVA for *B. subtilis* Experiment at 40 mJ/cm^2

Anova: Two-Factor With Replication

Source	SS	df	MS	F-ratio	*p*-value
Season(A)[a]	0.007	1	0.007	0.229	0.633
Homogenization (B)[b]	0.228	1	0.228	7.356	8.454×10^{-3}
A x B	0.003	1	0.003	0.112	0.738
Error	2.111	68	0.031		

[a] Season presented season water conditions; mid-winter and spring run-off.
[b] Homogenization represented Homogenization conditions; Homogenization before UV exposure and Homogenization after UV exposure.

Table D-12. Summary output of ANOVA for MS2 coliphage Experiment at 40 mJ/cm^2

Anova: Two-Factor With Replication

Source	SS	df	MS	F-ratio	*p*-value
Season(A)[a]	0.002	1	0.002	0.063	0.802
Blender(B)[b]	1.085	1	1.085	33.825	1.770×10^{-7}
A x B	0.025	1	0.025	0.807	0.372
Error	2.181	68	0.032		

[a] Season represented season water conditions; mid-winter and spring run-off.
[b] Blender represented blended conditions; blended before UV exposure and blended after UV exposure.

Table D-13. Summary output of ANOVA for *B. subtilis* Experiment

Anova: With Replication

Source	SS	df	MS	F-ratio	*p*-value
Sample [a]	0.141	3	0.047	0.099	0.960
Error	66.016	140	0.471		

[a] Sample presented different waters.

Table D-14. Summary output of ANOVA for MS2 coliphage Experiment

Anova: With Replication

Source	SS	df	MS	F-ratio	*p*-value
Sample [a]	0.036	3	0.012	0.043	0.987
Error	39.285	140	0.280		

[a] Sample presented different waters.

APPENDIX E BOX AND LUCAS CRITERIA

Since multi-target model is assumed to be the suitable model for *B. subtilis* spores UV inactivation process, the experiments have to be carried out in such a way that the two parameters of the model can be estimated precisely. As a non-linear model, the precision of the estimation comes from three factors: (i) the standard deviation from the experiment, (ii) the size of the sample at different factor levels, and (iii) the level of the factors.(Berthouex and Brown 2002) For the multi-target model, there are two parameters need to be determined, k_m and n_c. The precision of k_m and n_c estimation can be visualized by drawing the Joined Confidence Region (JCR). In even distribution along the UV dose experiment design, the JCR from k_m and n_c determination is of a elongated, 'banana' shape, which releases that the estimation of k_m and n_c are affected by each other. k_m and n_c are highly correlated. To improve the precision of the parameter estimation of the model, the correlation between the two parameters has to be reduced.

When least square method is used to estimate the model parameters, the variance and the covariance of the parameters is approximated as:

$$Var(b)=[X'X]^{-1}\sigma^2$$

where: b is the estimate of the model parameter using least square method, X is the derivative matrix from Taylor expansion of the model, X' is the rotated matrix of X. σ is the standard deviation of the measurement. Since σ is fixed in an experiment, Var(b) would have minimum value as long as determinate of $[X'X]^{-1}$ is minimum which means the determinate of [X'X] is maximum. This can be notated as:

$$\text{Max } \Delta=\max |X'X|.$$

In multi-model case, $\Delta=(X_{11}X_{22}-X_{12}X_{21})^2$, and let

$\Delta^* =\max|\, X_{11}X_{22}-X_{12}X_{21}\,|$.

Hence, the maximum absolute value of $X_{11}X_{22}-X_{12}X_{21}$ is used as a criterion for the minimum Var(b).

For multi-target model:

$$X_{1j}=\frac{\partial(1-(1-e^{\theta_1 t_j})^{\theta_2})}{\partial\theta_1}=\theta_2 t_j e^{\theta_1 t_j}(1-e^{\theta_1 t_j})^{\theta_2-1} \qquad j=1,2$$

$$X_{2j}=\frac{\partial(1-(1-e^{\theta_1 t_j})^{\theta_2})}{\partial\theta_2}=-(1-e^{\theta_1 t_j})^{\theta_2}\ln(1-e^{\theta_1 t_j}) \qquad j=1,2$$

$\Delta^*= \max|\, X_{11}X_{22}-X_{12}X_{21}\,|$

$$=\theta_2[t_2 e^{\theta_1 t_2}(1-e^{\theta_1 t_2})^{\theta_2-1}(1-e^{\theta_1 t_1})^{\theta_2}\ln(1-e^{\theta_1 t_1})-t_1 e^{\theta_1 t_2}(1-e^{\theta_1 t_1})^{\theta_2-1}(1-e^{\theta_1 t_2})^{\theta_2}\ln(1-e^{\theta_1 t_2})]$$

Here: $\theta_1=-k_m I$, I is the average fluent rate of the UV collimated beam.

$\theta_2=n_c$

k_m and n_c can be estimated before the experiment by referring to other researchers' data or by carrying out primary experiments. The optimal points of t_1 and t_2 can be computed by substituting km and nc value to the Δ^* formula . Excel 'solver' is set up to calculate the maximum value of the Δ^* value.

In my case, k_m=0.4, and n_c=8.5, and I =0.035 mW/cm^2

Then t_1=128 second, and t_2=240 second exposure time.

By adding more points at t_1 and t_2, the JCR can be improved. However, the improvement of the JCR strongly depends on two assumptions: (i) multi-target model is suitable for the *B. subtilis* spores UV inactivation modeling, and (ii) my k_m and n_c

values from former experiments are close to the 'real' values of the parameters. (Q Ke, 2006, Optimal Points for the inactivation test to estimate the multi-target model, Personal communication)